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THE RELATION OF THE BLUEGRASS BILLBUG, CALENDRA PARVULA (GYLLENHAL), TO THE DEVELOPMENT OF BASAL STEM ROT AND ROOT ROT OF CEREALS AND GRASSES IN NORTH-CENTRAL UNITED STATES[1]

E. W. Hanson, H. E. Milliron, and J. J. Christensen[2]

(Accepted for publication November 20, 1949)

Insects attacking the lower parts of plants frequently are an important factor in the development of basal stem and root rots of cereals and grasses. Heavy infestation of gramineous hosts is common in North-Central United States, and severe rotting of the basal parts of the plants is almost always associated with such insect injury (2, 3, 4). Several kinds of insects are involved, but certain species of billbugs are particularly important. These attack the lower internodes of stems, the crowns, and sometimes the roots, weakening the plants and providing avenues of entrance for rot-inducing fungi and bacteria. The billbugs characteristically fill the lower internodes with frass, which is a good medium for the rapid growth and multiplication of microorganisms.

Satterthwait (5) discusses 18 species of billbugs that attack cereals and grasses in the United States and says that *Calendra parvula* (Gyllenhal), the bluegrass billbug, probably ranks first in numbers and in destructiveness to farm crops. This species appears to be the most important in North-Central United States. The amount of infestation varies from year to year, locality to locality, and field to field.

The present investigation was made to obtain further information on the distribution, prevalence, and importance of the bluegrass billbug in relation to root and basal stem rots of cereals (particularly wheat) and grasses in North-Central United States.

SYMPTOMS OF INFESTATION

External symptoms of billbug injury seldom are conspicuous and can easily be overlooked. Severe root rot and, particularly, severe rotting of the bases of the stems, as indicated by discoloration and decay of these parts, are commonly associated with infestation (Figs. 1 and 2); premature "ripening" and breakage of the straw sometimes occur. These symptoms, however, do not necessarily indicate infestation, since they often develop on

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, and the Divisions of Entomology and Economic Zoology and Plant Pathology and Agricultural Botany of the Minnesota Agricultural Experiment Station. Paper No. 2494 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

[2] The writers are grateful to E. C. Stakman and A. A. Granovsky of the University of Minnesota for many helpful suggestions during the course of these studies, and to E. H. Herrling and A. J. Riker of the University of Wisconsin for assistance in the preparation of Figure 5.

plants not attacked by insects. Other, more reliable, external symptoms are the wounds—oviposition punctures and exit holes—produced by the insect on lower internodes of the culms (Fig. 1, A).

The best method of detecting billbug injury is to cut lengthwise the lower internodes of the culm. Infested parts usually are packed with castings of the larvae, a fine sawdustlike frass (Fig. 1, B). In early summer the eggs or larvae can be found inside the culms. Any number of culms of an individual plant may be infested; in some plants a single culm is attacked, in others all culms are infested. Any or all of the lower internodes of the culm may be attacked, but infestation most commonly occurs in one or two and does not extend throughout the culm. There is usually one larva

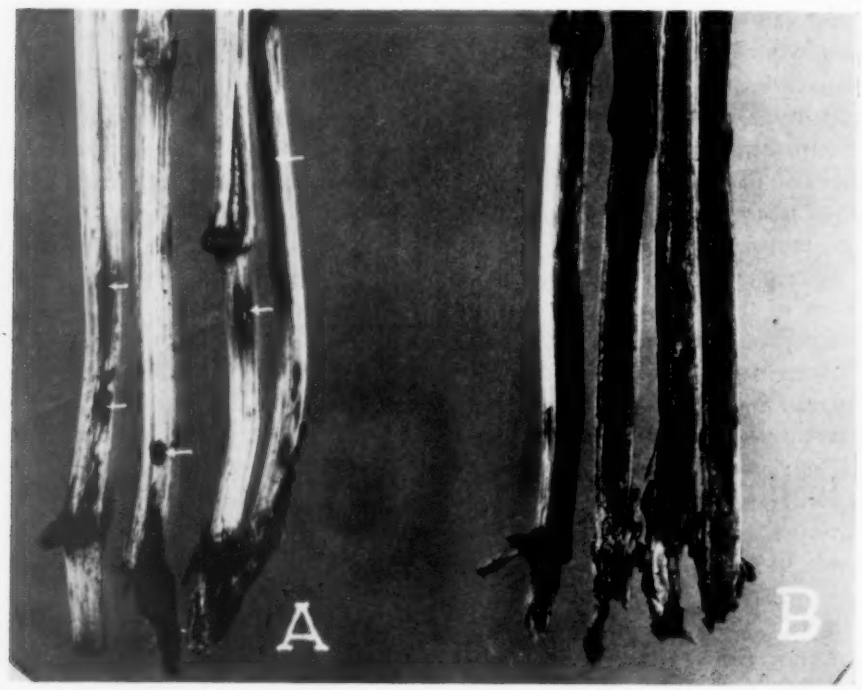

FIG. 1. Wheat culms with symptoms of billbug infestation. A. External symptoms are feeding and oviposition punctures made by the adults, exit holes made by the larvae, and usually considerable rotting of the infested internodes. Decay ordinarily starts around the wounds but eventually spreads throughout the infested internodes, giving them a brown discolored appearance. B. Culms split longitudinally to show the characteristic frass deposited by the larvae and the rotting associated with infestation.

in a given internode, but occasionally two or even three larvae. A larva is active within the internode in which it is hatched (Fig. 2, B), and only rarely does it penetrate to adjacent internodes (Fig. 2, F). It burrows downward (Fig. 2, A) or upward (Fig. 2, B) from the egg cavity and destroys the interior of the stem, leaving the wall so thin that it eventually may break. In cereals the larvae feed and complete most of their growth in the culms and emerge into the soil shortly before they are ready to pupate.

In this case little damage is done to the roots. The culms of many grasses, however, are too slender to permit the larvae to complete their development, so the larvae emerge before they are fully grown and feed upon the roots. The roots of *Poa pratensis* frequently are severely injured by the larvae.

LIFE HISTORY OF CALENDRA PARVULA

Satterthwait (5) studied the life history of many species of billbugs and stated that they ordinarily require one year to complete their development. The adult female hibernates, emerges in the spring, feeds, mates, and lays eggs. The eggs are usually deposited in tiny cavities cut into the stems of host plants. Egg laying occurs during about 2 months in late spring and

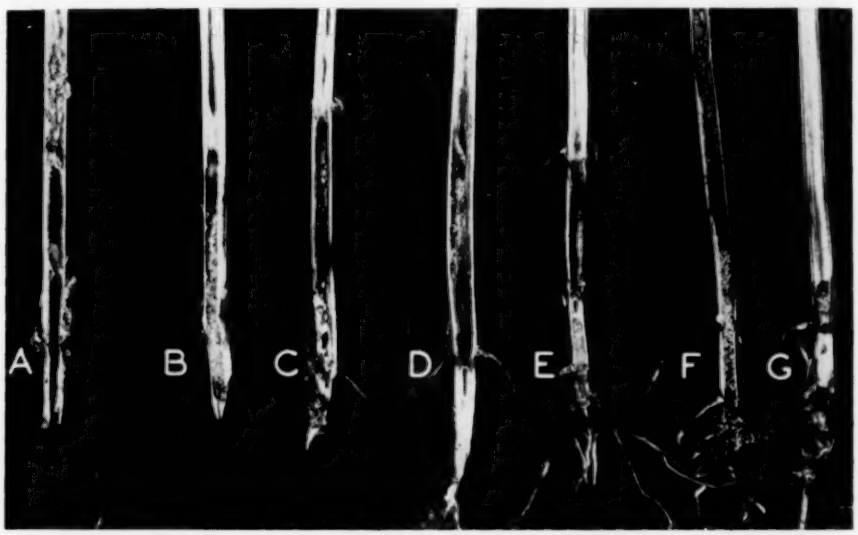

FIG. 2. Wheat culms infested by larvae of *Calendra parvula*. A. Larva that moved downward, reaming out the inside of the culm, leaving behind an abundance of frass. B. Larva that moved upward and was stopped by node. C. Severe decay in infested internode. D. Lower internode escaped infestation and decay while adjacent internode was infested and has begun to rot. E. Infested middle internode more severely rotted than adjacent noninfested internodes. F. Entire lower part of culm infested and in process of decay. G. Culm with exit hole produced by an emerging larva.

early summer. The larvae hatch 4 to 15 days after the eggs are laid and feed for several weeks before becoming fully developed and ready for pupation. Several days after pupation the pupa is transformed into an adult which may pass the winter in its pupal cell or may emerge and lead an active life for some time before becoming inactive for the winter. The larval stage of the bluegrass billbug is approximately 23 days, the pupal stage about 8 days, and the entire period from the laying of the egg to the appearance of the beetle is about 45 days. The writers have reared the bluegrass billbug, *Calendra parvula*, through its various stages and their observations concerning the life history agree with those previously published by Satterthwait. The various stages in the life history of *Calendra parvula* are shown in figure 3. The full-grown larva (Fig. 3, A) measures approxi-

mately 5 × 2.6 mm. in a natural, curved position, but its full length is about 6.8 mm. It is white except for the amber head. The pupa (Fig. 3, B, C, and D) measures approximately 6.8 × 2.7 mm.; during the first few days it is entirely white, but becomes dull brown prior to emergence of the adult (Fig. 3, E and F). The adult is about 7 mm. long and 2.4 mm. wide.

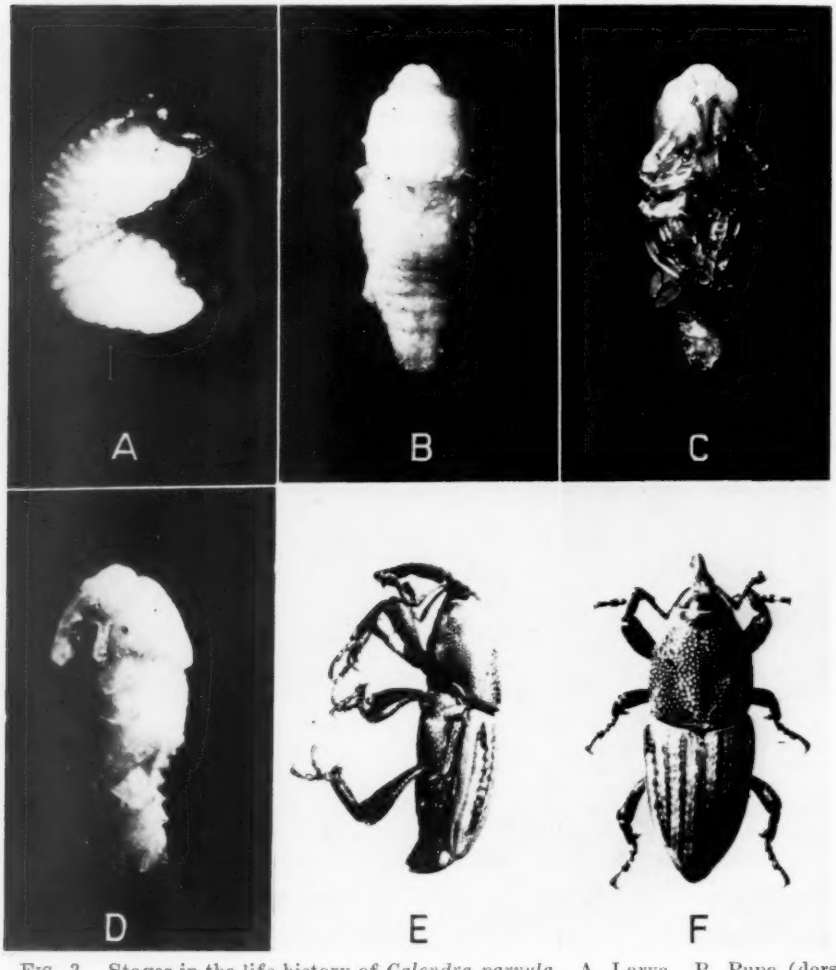

FIG. 3. Stages in the life history of *Calendra parvula*. A. Larva. B. Pupa (dorsal view). C. Pupa (ventral view). D. Pupa (lateral view). E. Adult (lateral view). F. Adult (dorsal view). Magnification 6×.

Newly emerged individuals are reddish brown but they become black after a few days. Body markings are illustrated.

DISTRIBUTION OF THE INSECT

According to Satterthwait (5), *Calendra parvula* "probably is distributed throughout the country. At least it is known from Canada to Florida and Texas and from the Atlantic Coast to South Dakota." The writers

have repeatedly found the insect in Minnesota, Iowa, Wisconsin, Illinois, Michigan, Missouri, and the eastern parts of North Dakota, South Dakota, Nebraska, and Kansas. Typically infested plants of several varieties of spring wheat have been obtained from fields as far west as Dickinson, North Dakota, and Bozeman, Montana. In the latter instances, however, no positive identification could be made because the insects had emerged from the plants when they were received. Although the writers have made adequate observations to ascertain that the species is generally distributed in the North-Central United States, most of their intensive surveys have been limited to Minnesota, northern Iowa, western Wisconsin, and the eastern parts of North Dakota and South Dakota. Further surveys are necessary in the remainder of the region before satisfactory estimates of the prevalence of the insect in those parts can be made. The data now available indicate that the insect is most prevalent in the southern half of Minnesota and that part of North-Central United States to the south and east. The amount of infestation is considerably less in the area north of that described. During the 8 years this study was in progress, infestations in northern Minnesota and eastern North Dakota were light in comparison with those farther south. The insect has not, in so far as the writers are aware, been reported in Canada. Very little is known about the importance of the insect in the western parts of the Dakotas, Nebraska, or Kansas. The meager data now available seem to indicate that the insect is not so prevalent in these areas as in the adjacent area to the east.

PREVALENCE AND HOST RANGE OF INSECT

In 1938, ninety per cent of the plants of several varieties of spring wheat were infested in some localities of southern Minnesota. In 1939 infestation was general throughout the North-Central United States and was found on many hosts, including both fall- and spring-sown wheat, barley, rye, *Agropyron repens* (L.) Beauv., *A. cristatum* (L.) Beauv., *A. smithii* Rydb., *A. pauciflorum* (Schwein.) Hitchc., *Agrostis alba* L., *Andropogon furcatus* Muhl., *A. scoparius* Michx., *Bouteloua curtipendula* (Michx.) Torr., *B. gracilis* (H. B. K.) Lag., *Bromus inermis* Leyss., *Elymus canadensis* L., *E. junceus* Fisch., *Festuca elatior* L., *Phalaris arundinaceae* L., *Phleum pratense* L., *Poa ampla* Merr., *P. compressa* L., *P. pratensis* L., *Stipa spartea* Trin., *S. viridula* Trin., and other grasses. The plants infested ranged from a trace to 96 per cent, with many of the hosts in the southern half of Minnesota and the eastern part of South Dakota averaging from 30 to 60 per cent. Infestation was very common in Iowa, western Wisconsin, and the rest of the region to the south, but the survey was not considered sufficiently extensive to permit drawing conclusions regarding the percentages of infestation in these parts. In the northern half of Minnesota and in North Dakota the infestation usually did not exceed 10 per cent on any host and commonly was less. The actual percentages of infestation for several varieties of spring-sown wheat for six locations in 1939 are given in table 1.

TABLE 1.—*Percentage of infested plants and culms of several varieties of spring wheat grown in different parts of Minnesota, North Dakota, and South Dakota in 1939*

Location	Variety	Percentage infested	
		Plants	Culms
St. Paul, Minn.	Mindum	82	74
	Thatcher	53	35
Waseca, Minn.	Ceres	30	17
	Marquis	21	11
	Mindum	41	31
	Thatcher	18	11
Crookston, Minn.	Ceres	16	7
	Marquis	5	2
	Mindum	6	4
	Thatcher	12	6
Fargo, N. D.	Marquis	1	1
	Thatcher	3	1
Dickinson, N. D.	Ceres	0	0
	Marquis	0	0
	Mindum	1	1
	Thatcher	0	0
Brookings, S. D.	Average for 3 hard red spring varieties[a]	33	12

[a] Identity of varieties not ascertained.

In 1940 billbug infestation was again found in all parts of the region. Intensive surveys were made, however, only in Minnesota and the eastern parts of the Dakotas. In southern Minnesota and South Dakota the prevalence of infested plants was as high as 70 per cent in some fields of spring wheat. In northern Minnesota and eastern North Dakota, infestation was low, generally less than 10 per cent. In 1941 a general survey could not be made, but approximately 25,000 plants of 4 varieties of wheat were collected from random fields in the vicinity of St. Paul, Minnesota. The culms were sectioned, and the results tabulated (Table 2). In 1942, a general survey again showed that billbugs were present in all but the northern part of the region. From 10 to 60 per cent of the plants of wheat, barley, rye, and many of the grasses in the southern half of Minnesota and the eastern part of South Dakota were infested. In west-central Minnesota, Ceres, Marquis,

TABLE 2.—*Billbug infestation in four varieties of spring-sown wheat collected from random fields near St. Paul, Minnesota, in 1941*

Variety	Plants		Culms	
	Number examined	Percentage infested	Number examined	Percentage infested
Ceres	7018	20	9228	17
Marquis	6423	20	8750	16
Mindum	5268	43	6477	39
Thatcher	6436	19	8906	15
Total or average	25145	26	33361	22

Mindum, and Thatcher wheats had 37, 15, 45, and 21 per cent, respectively, of the plants infested. Virtually no infestation was found in any host in northwestern Minnesota or northeastern and east-central North Dakota. The amount of infestation at St. Paul, Minnesota, and vicinity for various hosts in 1942 is indicated in table 3.

The percentages in table 3 do not necessarily indicate the relative differences between the various hosts in susceptibility to billbug attack. For example, in 1942 only 1 per cent of the culms of *Agropyron cristatum* were infested, notwithstanding the fact that in 1939 and 1940 some clones of this

TABLE 3.—*Billbug infestation on various cereal and grass hosts at St. Paul, Minnesota, and vicinity in 1942*

Host	Percentage infested	
	Plants	Culms
Cereals		
Barley	56	21
Oats	3	2
Rye	56	19
Wheat, Spring, Hard red	54	31
do Durum	70	48
do Winter, Hard red	48	13
Grasses		
Agropyron cristatum	—[a]	1
A. pauciflorum	–	31
A. repens	–	40
A. smithii	–	38
Agrostis alba	–	27
Andropogon furcatus	–	9
A. scoparius	–	0
Bouteloua curtipendula	–	7
Bromus inermis	–	19
Dactylis glomerata	–	0
Elymus canadensis	–	44
E. junceus	–	26
Festuca elatior	–	25
Panicum virgatum	–	0
Phalaris arundinacea	–	1
Phleum pratense	–	17
Poa ampla	–	23
P. compressa	–	7
P. pratensis	–	34
Stipa viridula	–	7

[a] No attempt was made to separate individual plants in the grasses.

host had 100 per cent of the culms attacked and killed by the insects and by the accompanying root and basal stem rots. Also, in Minnesota at least, barley and rye usually have lower percentages of infestation than spring-sown wheat.

In 1943, billbug infestation was general in distribution but the prevalence in most localities was considerably lower than in previous years. The average number of infested plants for spring-sown wheat was less than 15 per cent and for most hosts did not exceed 25 per cent. In the vicinity of St. Paul, however, the prevalence of infestation was relatively high, being 40 per cent in several fields of Mindum durum and 50 per cent in several

fields of fall-sown rye. The average percentages of infestation on four spring wheat varieties grown at University Farm, St. Paul, Minnesota, in 1943 are in table 4.

In 1944, billbug infestation was again general throughout the North-Central United States and was present in moderate amounts in many fields of southern Minnesota and that part of the region to the south. The average infestation was between 20 and 35 per cent for plants of spring wheats and much higher for some of the grasses, particularly the bluegrasses and timothy. The insect was present but did not cause important damage in northern Minnesota or in eastern North Dakota in 1944. In a field at St. Paul where wheat had been grown for 27 consecutive years the average infestation for 21 spring wheat varieties was 46 per cent of the plants and 28 per cent of the culms. In this study, 92 per cent of the plants and 83 per cent of the culms of the durum variety, Mindum, were infested. The average infestation for 5 varieties of barley was only 15 per cent of the plants and 11 per cent of the culms. No infestation was found in oats, the collections of which comprised several varieties but principally Vicland, Tama, and Minrus.

TABLE 4.—*Billbug infestation in four varieties of spring wheat grown in replicated plots at University Farm, St. Paul, Minnesota, in 1943*

Variety	Average percentage infested	
	Plants	Culms
Ceres	61	39
Marquis	30	12
Mindum	87	58
Thatcher	61	49

The 1945 survey was limited to Minnesota where there was only light infestation in the northern part but moderate amounts on some hosts in the southern part of the State. Table 5 gives the data for various hosts examined at St. Paul.

RELATIVE SUSCEPTIBILITY OF VARIOUS HOSTS TO CALENDRA PARVULA

Among the cereals grown in the region, wheat was most severely infested, followed by winter rye, barley, and oats. Of the wheats, the durums were most susceptible, followed by the hard red spring and hard red winter varieties. Only one variety of emmer, Vernal, was observed and it was almost as heavily infested as the durums. *Triticum timopheevi* Zhuk., now being used as a parent in some of the wheat breeding work in the region, was grown in small plots at St. Paul, Minnesota, for 5 years and not more than 4 per cent of the plants were infested. Since plots of this wheat were surrounded by plots of durum and hard red spring varieties having much higher percentages of infestation, it would appear that timopheevi wheat has considerable resistance to the insect. More extensive tests should be made, however, before definite conclusions are reached.

About 150 hard red spring and durum wheats were tested in an effort to find wheats resistant to the insect. None had a high degree of resistance. All of the durums now recommended in the area, including Mindum, Carleton, and Stewart, were susceptible. All of the hard red spring wheats tested were at least moderately susceptible, including such commercial varieties, or former commercial varieties, as Ceres, Marquillo, Thatcher, Mida, Pilot, Regent, Henry, Rival, Newthatch, and Marquis. Wheats such as Hope, and selections of Ceres-Hope-Florence × Thatcher, H44-Marquis × Thatcher, and Merit × Pilot, now used extensively in breeding programs, were susceptible to the insect. Progress and Haynes Bluestem which have been used to some extent as a source of resistance to Fusarial head blight

TABLE 5.—*Billbug infestation on various cereal and grass hosts at St. Paul, Minnesota, in 1945*

Host	Percentage infested	
	Plants	Culms
Cereals		
Barley (av. 5 varieties)	9	3
Oats (av. 4 varieties)	1	1
Rye (Emerald)	27	11
Spring wheat (av. 10 varieties)	41	16
Other field crops		
Alfalfa	0	0
Alsike clover	0	0
Flax	0	0
Potatoes	0	0
Red clover	0	0
Soybeans	0	0
Sweet clover	0	0
White clover	0	0
Grasses		
Agropyron cristatum	–[a]	2
A. repens	–	8
Bromus inermis	–	1
B. tectorum L.	–	14
Dactylis glomerata	–	1
Phalaris arundinacea	–	0
Phleum pratense	–	3
Poa pratensis	–	19

[a] No attempt was made to separate individual plants in the grasses.

were very susceptible. While varieties of a given class of wheat appeared to differ somewhat in susceptibility to the insect, it was difficult to rank them because insect infestation on a given variety fluctuated greatly with different conditions. No attempt was made to determine the relative resistance of varieties of hard red winter wheat, rye, or barley.

Winter rye is commonly infested; in some years as heavily as hard red spring wheat. On the average, barley is much less susceptible than either wheat or rye but occasionally it also becomes severely infested. In 1942, in one field of barley near Lamberton, Minnesota, 66 per cent of the plants and 27 per cent of the culms were infested; in another field near St. Paul, 56 per cent of the plants and 21 per cent of the culms were infested. These

percentages, however, are much higher than those usually found in barley. Oats are seldom attacked by *Calendra parvula*. During the 8-year period from 1938 to 1945 only a trace of infestation was found in any variety of oats, with the exception that in 1942 a few fields were found with as much as 9 per cent of the plants and 2 per cent of the culms infested. Satterthwait (5) states that the bluegrass billbug also attacks corn under some conditions. The present writers, however, during their limited observations, found no important damage to corn from this billbug. Young corn plants with leaf perforations similar to those described by Satterthwait as caused by billbugs were seen occasionally, but the damage could not be definitely ascribed to *C. parvula*.

Among grasses other than cereals, genera with the highest percentages of infestation were *Agropyron*, *Poa*, *Phleum*, *Elymus*, *Festuca*, *Agrostis*, and *Bromus*. There were differences in susceptibility not only between the various species in a genus but also between strains of a given species. For example, the average percentage of infested culms for various strains of *Poa pratensis*, grown in randomized plots, replicated three times, at St. Paul in 1945, ranged from 0 to 49, with 19 as the average for all strains.

EFFECT OF THE INSECT ON THE DEVELOPMENT OF ROTS

The parts of the plants infested by the bluegrass billbug are almost invariably more severely rotted than the corresponding parts of noninfested plants. To anyone familiar with the symptoms of infestation it seems almost superfluous to give data to demonstrate this fact. By the time infested plants are mature they almost always have at least a moderate amount of decay and commonly the infested parts are severely rotted. During the 8-year period from 1938 to 1945, several varieties of spring wheat were grown in replicated plots each year at St. Paul. A few days before the plants were mature, samples of about 600 plants from each plot were dug and separated into infested and noninfested lots. Each lot was then classified for severity of disease. The data in table 6 are representative of those obtained and suffice to illustrate the effect of the billbug on the severity of basal stem and crown rots. Similar observations were made on replicated plots at Hastings, Lamberton, and Waseca, Minnesota, as well as on numerous samples from farmers' fields throughout the North-Central United States. The effect of infestation on the development of rots in other hosts was similar to that found in wheat. Almost invariably the infested plants were more severely rotted than noninfested plants. Decay starts around the wounds produced by the adult in the process of feeding and depositing eggs (Fig. 4) and then follows the path of the larvae inside the culm. The organisms causing the decay increase on the moist castings deposited in the lower internodes and attack the walls of the culms from within. From infested tissues the decay organisms can spread to noninfested tissues so that late in the season it is not uncommon to find the entire base of the plant rotted even though some of the culms may not have been infested by the insect.

Although billbug infestation in the field is virtually always accompanied by rots, it would be interesting to know what proportion of the injury to plants is caused by the insect and what part is caused by rots. It should be possible to obtain this information if plants could be grown, with and without the insect, on soil infested with the rot-causing organisms and on soil free from such organisms. One attempt was made to conduct such a study with Ceres and Mindum wheat in the greenhouse in 1941; it failed because the larvae died a few days after hatching, apparently from unfavorable conditions within the cellophane cages placed over the plants to confine the insects. The adult billbugs, however, seemed to thrive in these enclosures: they could be seen feeding on the stems of the plants;

TABLE 6—*The effect of billbug infestation on the severity of basal stem and crown rots of four varieties of spring wheat grown at St. Paul, Minnesota, in 1938, 1942, and 1944*

Year and variety	Level of disease[a]	
	Noninfested plants	Infested plants
1938		
Ceres	L	H
Marquis	L–	H
Mindum	L+	H
Thatcher	T+	H
Average	L–	H
1942		
Ceres	L+	H
Marquis	L	H
Mindum	M	H
Thatcher	L	M
Average	L+	H–
1944		
Ceres	M	H
Marquis	L	H–
Mindum	M	H
Thatcher	L–	H–
Average	L+	H–
Average	L	H–

[a] Basal stem and crown rot ratings were based on five severity classes: no infection, plants clean (C); trace (T); light (L); moderate (M); and heavy (H). + = more severe; – = less severe. No variety was free from infection.

they mated and laid numerous eggs; the eggs hatched, and the young larvae began feeding within the stems in the usual manner. Since the larvae died before they could cause appreciable injury to the plants, it was not possible to obtain conclusive information. The writers had no opportunity for further study of this phase of the problem. Without doubt, however, the insects themselves can and do damage the host plants. They may chew the root system in some of the grasses, such as the Poas, so completely as to kill the plants, particularly under arid conditions. Foot and root rots alone, of course, can and frequently do kill host plants, but they are much more severe when aided by the insects (Table 6). The earlier the plants

are attacked by the larvae, the greater the loss. In the North-Central United States, however, by the time the larvae become abundant and the billbug-rot combination becomes very destructive, the plants have reached the boot stage and most plants are able to produce a fair yield of seed. No experiments were made to measure the billbug-rot effect on yields, but considerable shriveling of the kernels of infested plants is common, as compared with kernels of noninfested plants from the same plots. In old stands of perennial grasses, such as timothy or bluegrass, plants are frequently killed or severely injured.

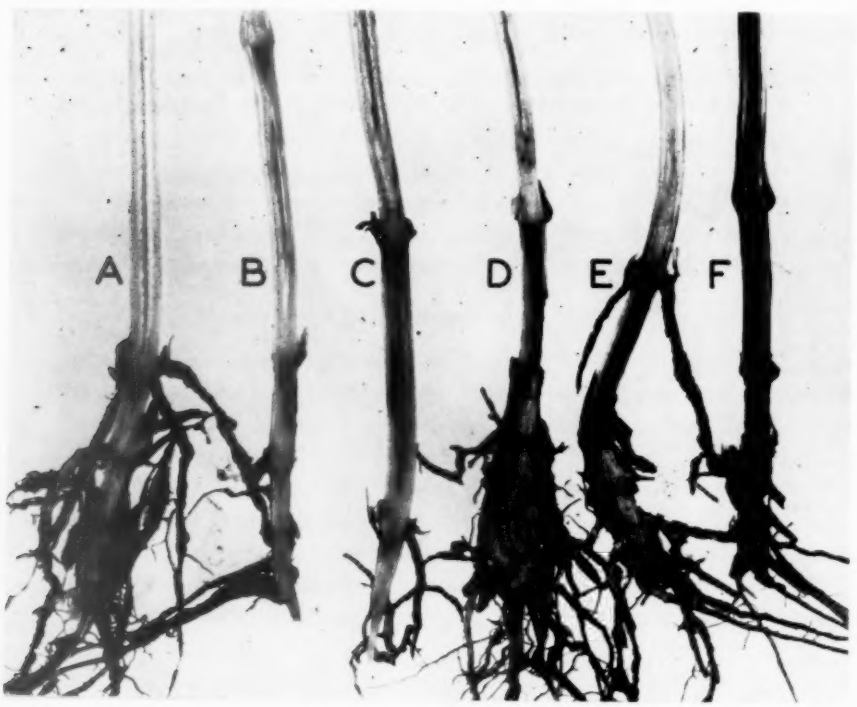

FIG. 4. Wheat culms split longitudinally to show that insect wounds serve as avenues of entrance for rot-inducing organisms and facilitate decay. A. Noninfested, healthy culm. B, C, D, and E. Decay is localized at first around insect wounds and spreads most rapidly through infested areas. F. Lower internodes of infested culm completely destroyed by rots.

MICROFLORA ASSOCIATED WITH BILLBUG INFESTATION

The kind and relative prevalence of the various microorganisms associated with the rots of billbug-infested plants are usually similar to those associated with the rots of noninfested plants in the same stage of development, except that a higher percentage of weak pathogens and saprophytes are present in the parts of the plants that have been invaded by the insect. The kind and relative prevalence of these microorganisms vary considerably with the locality, climatic conditions, previous treatment of the land, host, stage of development of host, and many other factors. This phase of the

problem cannot be discussed comprehensively here. However, lest the writers' concept of the causes of foot and root rots be misunderstood, it should be stated that their observations indicate that these rots, and also the seedling blights, are caused not by one organism but by a complex of soil-inhabiting microorganisms which is influenced by a multiplicity of factors. Intensive studies by the writers relative to the microflora associated with foot and root rots have been limited to Minnesota and the eastern parts of North and South Dakota, but in this area *Helminthosporium sativum* P., K., and B. has usually been the most important member of the complex causing root and basal stem rots of wheat, and *Pythium arrhenomanes* Drechs. and *H. sativum* have been the most important of these pathogens on the forage grasses. These observations agree closely with those reported by Sprague (6) for North Dakota. As mentioned, *Calendra parvula* does most of its damage in North-Central United States after the plants are well advanced in development and are approaching the heading stage. The insect has not, therefore, been an important factor in the development of seedling blights and foot and root rots occurring in the spring. It aids particularly the organisms that are active during the summer months and, in the case of the grasses, the fall months.

From 50 to 85 per cent of all the isolates from either infested cereals or forage grasses usually were *Fusarium* spp. and the prevalence of *Fusarium* increased as the plants approached maturity. The relative prevalence of *Helminthosporium* was high on some hosts such as wheat in the earlier stages of infestation, but gradually declined as the prevalence of *Fusarium* increased. Several kinds of bacteria also were present in infested plants. A great many of the soil-borne organisms were found inside the stems in the larval castings which seemed to favor the rapid spread and multiplication of fungi. Both larvae and adults carry fungi and bacteria on and within their bodies. Many different microorganisms were isolated from these insects; *Fusarium* was most commonly obtained, but *Helminthosporium*, *Alternaria*, *Penicillium*, *Mucor*, *Epicoccum*, *Colletotrichum*, and others also were isolated.

CONTROL OF THE INSECT

The bluegrass billbug does not migrate very rapidly and consequently can be controlled effectively by good cultural practices. Suitable crop rotation including the use of such apparently immune crops as alfalfa, red clover, alsike clover, white clover, Ladino clover, sweet clover, soybeans, flax, and Irish potato greatly reduces the amount of infestation. Growing susceptible crops on the same land for several consecutive years should be avoided. Also, susceptible crops should not be grown on newly plowed timothy fields, bluegrass pastures, or other grasslands without first growing a nonsusceptible crop. Susceptible grasses along fences, roads, railroad rights of way, and the like, are a source of infestation and should be reduced to the minimum. Clean cultivation and fall plowing are beneficial.

Satterthwait (5) points out that many kinds of birds feed on billbugs and should be protected. He suggested community action in adopting control measures.

An effort was made to determine the possibilities of reducing the amount of infestation on spring wheats by manipulation of the time of seeding. From 1940 to 1945, inclusive, several seedings were made at St. Paul, Minnesota, at intervals of approximately 1 week, beginning as early as it was possible to work the land in the spring and continuing until about the first part of June. No early seedings could be made in 1942 or 1944 because of excessive rainfall during the spring season. Each seeding was replicated four times and included from four to five varieties. Mindum and Thatcher were used each year. In 1940, 1941, and 1944 the varieties Ceres and

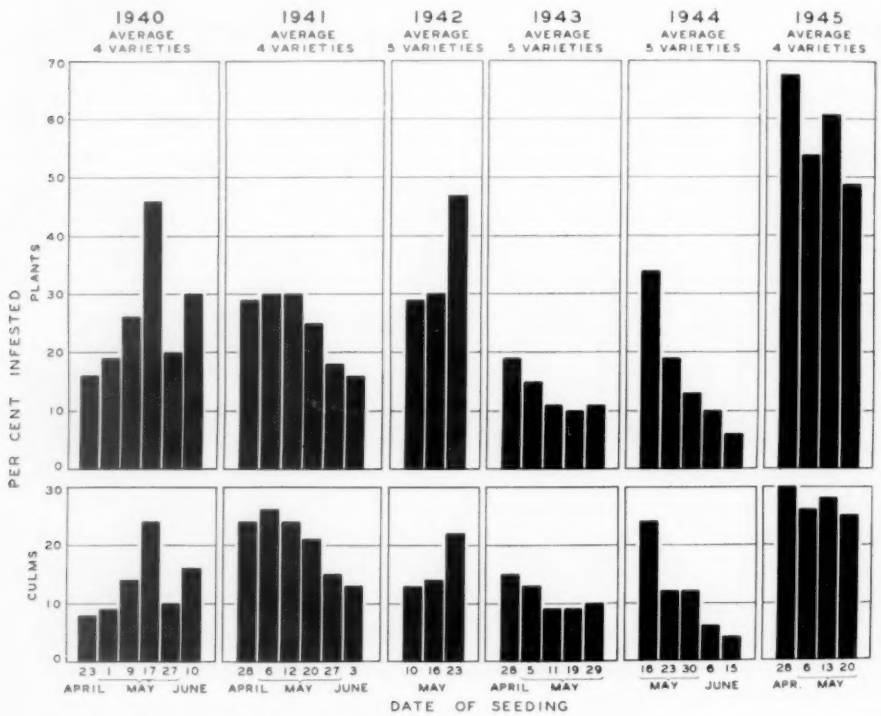

FIG. 5. The effect of time of seeding spring wheats on the percentages of plants and culms infested by the bluegrass billbug at St. Paul, Minnesota, 1940 to 1945.

Marquis were included; in 1942 and 1943 Hope, Kota, and Progress were sown; and in 1945, Hope and Reward. All plants were examined at the mature stage to permit maximum infestation. About 800 plants of each variety for each date of seeding were examined each year and the number of culms observed in each case ranged from 1200 to 1800. The results (Fig. 5) show that while there were great differences in amounts of infestation in plots sown at different dates, the effect was not consistent from year to year. For example, in 1940 the earliest seeding had the least in-

sect injury and the amount of infestation increased progressively in subsequent seedings up to May 17; in 1945 an opposite trend was found, the earliest seeding having the greatest amount of infestation. In 1941, 1943, and 1944 the latest seedings had the least infestation; but it is not practical to delay seeding spring wheat in North-Central United States until the last week of May or the first part of June. On the contrary, it is a well-established fact that in general the earlier the seeding date in this area the better the yield. The data obtained to date do not indicate that the insect can be satisfactorily controlled, in spring wheats at least, by any manipulation of the time of seeding.

DISCUSSION

The bluegrass billbug is widely distributed in North-Central United States, and because it attacks the lower internodes of the stems and the roots of cereals and many grasses it sometimes is an important factor in the development of root rots and basal stem rots. The insect not only provides avenues of entrance for soil-borne pathogens but carries them on and in its body into the wounds. It also weakens the plants by its feeding. The role of the billbug would be very important, indeed, if it were not for the fact that the larvae usually do not appear until the plants are well advanced in development. Thus such short-lived hosts as the cereals usually can mature seed before they are killed by the rots that accompany infestation. The earlier the infestation, other factors being equal, the greater is the severity of the rots and the greater the damage to the plants. Reductions in yield and quality of grain have not been measured, but premature killing of plants, breaking and lodging of the culms, and shriveling of the kernels have been observed in both plots and fields where infestation was present. In perennial grasses the insects and rots have a greater opportunity to cause severe injury because they are not limited by the time factor as they are in the case of the cereals which mature early and are harvested by the middle of the summer. In the grasses, the insects and particularly the pathogens continue their activities until late in the fall and sometimes cause considerable loss in hay and pasture as well as premature failure of the sod.

Since the billbug is commonly found in the grasses along roadsides, fence rows, railroad rights of way, and in old timothy fields and bluegrass pastures, it is important, in making surveys to estimate billbug prevalence, to include observations a considerable distance from these sources of infestation. The margins of any field of a susceptible crop are likely to become infested if they adjoin such sources of infestation. As an observer walks farther into a field the prevalence gradually decreases if good crop rotation has been practiced. Any survey in which an observer takes all of his samples within close proximity of the highways is likely to give too high an estimate.

While *Calendra parvula* appears to be the most important billbug on cereals and grasses in North-Central United States, it is not the only one. *Anacentrinus deplanatus* (Casey) has been found throughout the area on

Echinochloa crusgalli (L.) Beauv. (1, 3), and Satterthwait (5) describes several other species of billbugs occurring in the area, some of which are very destructive to corn as well as cereals and grasses. Billbugs and other insects that attack the lower internodes of stems or the roots of host plants render them more vulnerable to attack by rot-inducing microorganisms.

SUMMARY

Calendra parvula, the bluegrass billbug, was found widely distributed in North-Central United States, particularly in Minnesota, Wisconsin, Iowa, Illinois, Michigan, Missouri, and the eastern parts of North Dakota, South Dakota, Nebraska, and Kansas.

The prevalence and importance of the insect vary greatly with locality, host, previous cropping practices, and other factors. It has a wide host range among the cereals and grasses. Spring and winter wheats, rye, and barley are commonly attacked while oats are only occasionally infested. Among the grasses other than cereals, *Agropyron, Poa, Phleum, Elymus, Festuca, Agrostis,* and *Bromus* have been the genera with the highest percentage of infestation. Differences in susceptibility were found not only between the various species in a genus but also between varieties and strains of a given species.

The insect itself is destructive; in addition it may enhance the development of pathogens responsible for basal stem and root rots of cereals and grasses. It attacks the lower internodes of the stems and sometimes the roots, weakening the plants and making them more vulnerable to attack by soil-borne pathogens. It provides avenues of entrance for these pathogens, and both adults and larvae of the insect carry fungi and bacteria on and in their bodies. Infested plants almost invariably are more severely rotted that noninfested plants.

The microflora associated with the rots of infested plants is usually similar to the microflora of noninfested plants in the same stage of development, except that a higher percentage of weak pathogens and saprophytes are present in the parts of the plants that have been infested. The kind and relative prevalence of these microorganisms vary considerably with the locality, climatic conditions, host, stage of development of host, cultural practices, and many other factors.

The insect can be controlled satisfactorily by good cultural practices. Suitable crop rotation, including the use of such apparently immune crops as alfalfa, clover, soybeans, flax, or Irish potato, will greatly reduce the amount of infestation. Growing susceptible crops on the same land for several consecutive years should be avoided. Also, susceptible crops should not be grown on newly plowed timothy fields, bluegrass pastures, or other grasslands without first growing a nonsusceptible crop. Susceptible grasses along roads, fences, ditches, railroad tracks, and the like are commonly infested and should be reduced to a minimum. Date of seeding influenced the amount of infestation in spring wheats at St. Paul, Minnesota, but the re-

sults were not consistent from year to year and consequently it does not seem that any manipulation of the time of seeding will be effective in controlling the insect.

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STUDIES ON ANTIBIOTIC SOIL ORGANISMS. I. ACTINOMYCETES ANTIBIOTIC TO PYTHIUM ARRHENOMANES IN SUGAR-CANE SOILS OF LOUISIANA

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It is well known that many microorganisms in the soil are antibiotic. It has been assumed also that some of these play a role in controlling soil-borne pathogens since frequently it has been observed that pathogenic organisms cause more damage when introduced into sterilized soil than into unsterilized soil.

In the sugar belt of Louisiana, one of the important soil-borne pathogens is *Pythium arrhenomanes* Drechsler (3, 8), which under certain conditions causes a severe root rot of sugar cane. It was felt that a study of the microorganisms present in the different soils of Louisiana which were antibiotic to this *Pythium* might yield information as to the role played by microorganisms in reducing root rot losses. As certain Actinomycetes were known to be antibiotic to *Pythium* (9), and to occur in considerable numbers in sugar-cane soils (1), this group was selected for the initial part of the study. This paper reports the results of a survey made over a period of 2 years, and includes an analysis of some of the factors responsible for the number and distribution of antibiotic Actinomycetes in the various soils.

SOIL COLLECTIONS

The soils in Louisiana on which sugar cane is grown for the manufacture of sugar are largely of alluvial origin. They have been classified by O'Neal and Hurst (7) into five major types or series and several subtypes. In the survey, samples were collected from important areas representing several of the soil types. The soils from which samples were collected include:

1. Yazoo: first bottom soils of the Mississippi alluvium. These soils are predominantly very fine sandy loams and silt loams, fairly well drained, with a pH predominantly within the range of 6.5 to 7.5. Samples were taken at eight locations.

2. Sharkey: first bottom soils of the Mississippi alluvium. These soils are predominantly clay and the drainage in general is poor. The pH ranges from 6.0 to 7.0 or slightly above. Samples were taken at seven locations.

3. Lintonia and Olivier: terrace soils. These are fairly well drained. The pH ranges from 5.0 to 6.0. Samples were taken at two locations.

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4. Iberia: silt loams of the coastal prairie. These soils are fairly well drained. The pH ranges from 6.0 to 7.0. Samples were taken at three locations.

5. Yahola: soils of the Red River area. These are very fine sandy loams and are well drained. The pH ranges from 6.7 to 8.3. Samples were taken at two locations.

MATERIALS AND METHODS

Soil samples were collected during two growing seasons and were stored in the laboratory in paper sacks until dilution cultures could be made. Usually, they were cultured within a week after collection.

After passing each sample through a 4-mesh screen, a 100-gm. portion was added to a flask containing 1 liter of water drawn from a hot water faucet but cooled to room temperature. Each flask was shaken periodically for approximately 3 hr. Following a vigorous agitation, 1 ml. of suspension was withdrawn and added to 100 ml. of sterile water in a plugged flask. This was further diluted to 1:100,000. One ml. of this dilution was thoroughly mixed with approximately 15 ml. of melted Conn's glycerol asparaginate agar (4) and poured into a sterile Petri dish. From five to seven dilution plates were poured from each soil sample and were incubated at 28° C. for 8 to 10 days. The number of Actinomycete colonies in each plate was then recorded and all individual colonies were transferred to oatmeal agar slants.

The test for antagonism against a pathogenic isolate of *Pythium arrhenomanes* was made by streaking four of the Actinomycete isolates about the edge of a 10-cm. Petri dish on Czapek's sucrose nitrate agar medium (4). After incubating for 48 hr. at 28° C. a circle of agar 7 mm. in diameter from an actively growing culture of *P. arrhenomanes* was placed in the center of the plate. This procedure allowed approximately 25 mm. between each Actinomycete culture and the *Pythium* inoculum. After incubation for 7 days at 28° C. the distance from the foremost edge of the *Pythium* culture to the center of each Actinomycete culture was determined and recorded in millimeters. Nonantibiotic Actinomycetes overgrown by the *Pythium* were discarded without further testing. Antibiotic cultures were retested. Any Actinomycete with an inhibition of 10 mm. in either of these two tests was again tested by streaking a single isolate in a Petri dish and placing the *Pythium* inoculum about 50 mm. from it. The culture medium, incubation time, and temperature were the same as in preliminary tests. Antibiotic activity of several Actinomycetes is shown in figure 1.

To compare the Actinomycete population associated with the sugarcane roots with that in the surrounding soil, cane roots were collected with the soil sample. Just before making the dilutions, the roots were separated from the soil with a 4-mesh screen. All the soil that could be shaken or easily rubbed off was removed. Dilution cultures were made from this soil

and also from the macerated roots. Dilutions as high as 1:10,000,000 were used with the roots. The same isolation and testing procedures were used as for the soil isolates.

Two arbitrary terms, "*antibiotic index*" and "*antibiotic value*," describe the nature of the Actinomycete populations. The antibiotic index is defined as the calculated mean inhibition of all Actinomycetes tested from any soil sample. The individual isolates were placed in one of five classes: 0, 1–5, 6–10, 11–15, and 16 or more millimeters inhibition. To simplify calculations, 0, 3, 8, 13, and 18, respectively, were considered as class means. The sum of the products of the frequency and class means, divided by the

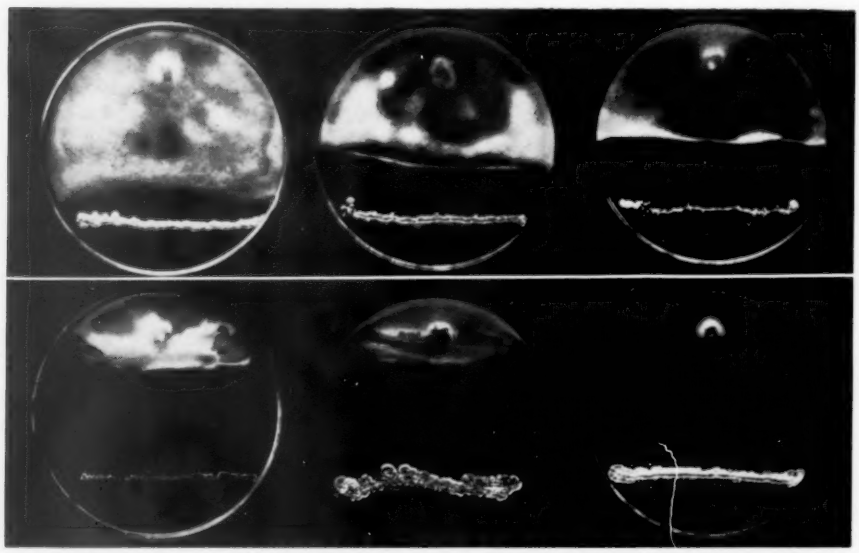

FIG. 1. Strains of Actinomycetes from sugar-cane soils antibiotic to *Pythium arrhenomanes*.

total number of isolates tested, gave the antibiotic index of the sample. As the number of isolates tested was not always proportional to the size of the population, the antibiotic value was devised as an adjustment for the difference. The antibiotic value was calculated for each soil sample by multiplying the antibiotic index by the number of thousands of Actinomycetes per gram of soil. The antibiotic value was considered to be a quantitative measure for the antibiotic activity of the Actinomycetes in any given soil.

RESULTS

As expected, the number of Actinomycetes in the soil samples varied considerably. Samples were collected at different seasons and from different types of soils, and the number of cultures from each sample was relatively small. The calculated number of Actinomycetes in a gram of soil varied from 135,000 to 4,375,000. While this variation made compari-

son somewhat difficult, it is believed that a sufficient number of determinations was made to yield statistically reliable data.

An analysis of variance of the individual plate counts was calculated for 135 soil samples. This included only those samples from which the colonies developing in four Petri plates were counted. The mean population of these samples was 1,634,000 Actinomycetes per gram. The least significant difference between any two populations was found to be 535,000 Actinomycete colonies per gram of soil. Although this value is rather large, there were significant differences between some of the populations of individual soil samples.

Comparisons of Actinomycetes by Isolation Periods

The soil collections were made at various times during 1946 and 1947. These two years were unlike with respect to rainfall, 1946 being very wet in the early part of the year and 1947 being very dry from April through the greater part of September. In order to obtain information on the effect of weather and seasonal conditions on the Actinomycete populations, the results of the isolation work during four periods were considered separately. These four periods were: March 1 to June 1, 1946; June 1 to September 1, 1946; January 28 to June 1, 1947; June 1 to November 1, 1947. One soil type, Yahola, was not sampled in the first of these periods. The data obtained from the isolation work are summarized in table 1. Certain trends are indicated which may or may not be of significance.

As to the Actinomycete population, the Yazoo, Sharkey, and Iberia soils showed a common trend; the number of Actinomycetes increased from the first through the third period but declined sharply in the fourth. The Yahola soil showed slight decrease from period to period; the Lintonia and Olivier soils showed a more seasonal trend, with a decrease from spring to summer during both years.

The trends with respect to the antibiotic indices were not very pronounced. In general, this index for all soils was high in the second period and dropped in the third period. The index for Iberia soil seemed to be the most stable, changing only slightly from period to period.

As the antibiotic value is the product of the number of Actinomycetes and the antibiotic index of the population, it could be affected by factors influencing either the antibiotic index or the number of Actinomycetes in the soil. In all soils studied, there was a marked increase in antibiotic values from the first to the second period, and a decrease from the third to the fourth period. From the second to third period, two of the soils, Yazoo and Iberia, showed a marked increase, whereas the Yahola and the Lintonia and Olivier soils showed a very marked decrease. The value for Sharkey soil, on the other hand, changed very little during this period. The factors responsible for this divergent action of the Actinomycetes of the different soils are not evident, as all except Sharkey soil were fairly well drained loams. The two soils in which antibiotic value increased are al-

TABLE 1.—*Numbers, antibiotic indices, and antibiotic values of Actinomycetes from sugar-cane soils of Louisiana in relation to Pythium arrhenomanes*

Soil type	Period	No. of soil samples	No. of Actinomycetes per gm. of soil, in thousands	Antibiotic index	Antibiotic value
Yahola	1	0	.....	.....	.....
Do	2	4	1,431	3.76	5,088
Do	3	4	1,407	1.93	2,335
Do	4	10	1,080	1.78	2,009
Average			1,306	2.49	3,144
Yazoo	1	15	799	2.12	1,664
Do	2	12	1,417	2.48	3,368
Do	3	11	2,253	2.13	4,575
Do	4	7	1,251	1.33	1,653
Average			1,430	2.02	2,815
Sharkey	1	16	698	1.30	970
Do	2	9	1,486	2.20	2,942
Do	3	11	1,902	1.81	2,843
Do	4	6	813	1.38	1,409
Average			1,225	1.62	2,041
Lintonia & Olivier	1	3	1,214	1.45	1,739
Do	2	2	750	2.36	2,556
Do	3	6	1,467	0.95	1,534
Do	4	8	1,103	1.22	1,476
Average			1,134	1.49	1,826
Iberia	1	15	1,318	1.24	1,547
Do	2	4	1,369	1.54	2,144
Do	3	8	2,562	1.25	3,344
Do	4	6	1,081	1.37	1,644
Average			1,582	1.39	2,170

luvial soils, while those in which it decreased, although alluvial, were more mature formations, one with acid and one with neutral reaction.

Effect of Soil Reaction on the Actinomycete Population and Its Antibiotic Activity

The relationship between the soil reaction (pH) and the Actinomycetes with respect to number per gram of soil, the antibiotic index, and the antibiotic value is presented in table 2. No significant trend was observed in

TABLE 2.—*The relation of the Actinomycete populations, antibiotic index, and antibiotic value to soil reaction*

pH class	No. of soil samples	Antibiotic index	No. of Actinomycetes per gram of soil, in thousands	Antibiotic value
5.25	14	0.98	1,107	1,085
5.75	20	1.47	1,682	2,473
6.25	46	1.47	1,452	2,034
6.75	58	1.82	1,403	2,553
7.25	26	2.00	1,390	2,780
7.75	14	1.77	1,297	2,296
	Total 178	Av. 1.58	Av. 1,388	Av. 2,204

the number of Actinomycetes as the pH of the soil samples increased from 5.25 to 7.25, with perhaps the exception of the change from 5.25 to 5.75 pH. The antibiotic index increased consistently as the pH increased from 5.25 to 7.25 and then dropped at 7.75 pH. When the coefficient of correlation was calculated, no correlation was observed between the pH and the size of the Actinomycete population. The correlation, however, between the pH and the antibiotic index was highly significant for the light soils and significant for the Sharkey soils. There was an increase in the antibiotic index as the pH increased. Except in the lowest pH class, the antibiotic values showed no definite trends since the population tended to decrease as the antibiotic index increased. Thus the mean antibiotic activity of the individual Actinomycete increased with alkalinity of the soil, but the antibiotic potential of the total population remained about the same.

TABLE 3.—*The effect of rainfall on the Actinomycetes*

Population characteristic	Comparative rainfall		
	Low	Normal	High
		Light soils	
No. of comparisons	19	17	16
Millions per gram	1.04	1.59	1.88
Antibiotic index	1.87	1.89	1.82
		Heavy soils	
No. of comparisons	3	7	8
Millions per gram	0.70	1.24	1.80
Antibiotic index	1.17	1.14	1.14

Effect of Rainfall on the Actinomycete Population

In order to determine the effect of the rainfall upon the Actinomycete population the daily rainfall at the Cinclare, Reserve, Franklin, Jeanerette, Lafayette, and Cheneyville weather stations (5) were considered as representative for those particular areas. The samples from these locations were classified according to the rainfall during 2 weeks preceding sampling; 0–1.0, 1.1–3.0, and greater than 3 inches represent dry, normal, and wet conditions respectively.

The mean number and antibiotic index of the Actinomycetes from the soils in each class are shown in table 3. The number of Actinomycetes increased with the amount of rainfall in both light and heavy soils. The antibiotic index was not influenced by rainfall.

Actinomycetes Associated with Sugar-Cane Roots

A comparison was made of the Actinomycetes associated with sugar-cane roots and those in the surrounding soil. In the five soil samples used (Table 4) there were from 2.8 to 33.5 times as many Actinomycetes on the roots as in the surrounding soil. The antibiotic indices were very similar for the

TABLE 4.—*Actinomycetes on sugar-cane roots as compared to those in surrounding soil*

Soil type	No. per gm. soil (in thousands)			Antibiotic index		Antibiotic value	
	Roots	Soil	Root/soil ratio	Roots	Soil	Roots	Soil
Yahola	26,800	1,100	33.5	2.00	1.89	77,280	2,079
Do	23,268	1,500	28.6	1.38	1.49	31,877	1,213
Yazoo	4,143	1,500	2.8	1.74	1.79	7,209	2,685
Iberia	11,048	2,257	4.9	0.81	1.54	8,949	3,476
Lintonia & Olivier	10,000	875	11.4	0.79	0.62	7,900	540
Average	15,052	1,446	13.0	1.34	1.45	26,643	1,999

Actinomycetes from the two sources. Because of the large populations, the antibiotic values in the immediate vicinity of the roots were very high.

Degree of Antibiotic Activity in Isolates

In addition to determining the number of antibiotic organisms in the soil, it seemed important to determine the relative abundance of those having different degrees of antibiotic activity. Such information was secured by grouping isolates according to the distance in millimeters at which the growth of *P. arrhenomanes* was inhibited. The distribution of 8302 cultures isolated from 181 soil samples is shown in table 5.

The Yahola soil had the greatest percentage of antibiotic Actinomycetes, followed by the Yazoo, Sharkey, Lintonia and Olivier, and Iberia soils. In general, there was a close agreement between the percentage of antibiotic cultures and the antibiotic index.

DISCUSSION

The number of Actinomycetes per gram of soil found in this survey (1,388,000) agrees rather closely with that (1,280,000) found by Abbott (1) in two soils of the Yazoo type and one of the Terrace type in 1925. Apparently there has been little change in the number of Actinomycetes per gram of soil in the past twenty years as measured by the technique used.

TABLE 5.—*Percentage of isolates of Actinomycetes in each inhibition class antibiotic to Pythium arrhenomanes in Louisiana sugar-cane soils*

Soil type	No. of samples	Inhibition classes—millimeters								Antibiotic index
		0	1–5	6–10	11–15	16–20	21–30	31–40	41–	
Yahola	18	68.24	15.99	9.76	4.29	1.28	0.43	0.00	0.00	2.49
Yazoo	45	71.26	13.49	8.75	4.71	1.07	0.42	0.28	0.05	2.02
Sharkey	42	79.55	8.90	5.85	3.34	1.35	0.76	0.23	0.00	1.62
Lintonia & Olivier	18	78.13	14.28	3.67	2.65	0.88	0.38	0.00	0.00	1.50
Iberia	36	81.49	8.61	5.33	3.51	0.62	0.12	0.12	0.00	1.35
Others	22	81.12	7.53	7.04	2.54	1.47	0.29	0.00	0.00	1.44
Average		76.60	11.17	6.87	3.67	1.13	0.41	0.14	0.01	1.74

The proportion of cultures (23.4 per cent) antibiotic to *Pythium arrhenomanes* is comparable to that reported by Meredith and Semeniuk (6) for Actinomycetes in certain Iowa soils. These investigators reported that 21 per cent of the Actinomycete cultures isolated from the Iowa soils were antibiotic to *P. graminicolum* Subra.

The significance of the differences among the soil types with respect to the percentage of Actinomycetes antibiotic to *P. arrhenomanes* cannot be stated at present. It is of interest, however, to observe that the highest yields of sugar cane in varietal test plots have occurred in the Yahola soils, next in the Yazoo soils, while the Iberia, Terrace, and Sharkey soils have given the lowest yields of sugar cane (2). While further studies are necessary to determine the full significance of this correlation between the antibiotic potential of the Actinomycetes in the soil and sugar-cane yields, root rot caused by *P. arrhenomanes*, a limiting factor in sugar-cane production in Louisiana, is known to be less severe in the Yahola and Yazoo soils.

SUMMARY

During 1946 and 1947 a preliminary survey was made to secure information in regard to the Actinomycete population of the sugar-cane soils of Louisiana. This included not only a determination of the number of Actinomycetes present, but also a study of the antibiotic activity of the different Actinomycetes against a common root rot fungus, *Pythium arrhenomanes*.

The number of Actinomycetes per gram of soil as determined by dilution cultures varied from about 100,000 to 4,000,000 with an average of approximately 1,300,000.

Based on 8302 cultures isolated from 181 soil samples from five soil types, the percentage of isolates antibiotic to *P. arrhenomanes* ranged from 18.5 to 31.5 per cent for the different soils. Antibiosis was determined by the ability of each isolate to inhibit the growth of the fungus in Petri plates. Inhibition was evaluated in terms of the distance from the Actinomycete culture that the growth of *Pythium* was inhibited. Such inhibition varied from a trace to 41 mm.

The number of Actinomycetes per gram of soil was not significantly different in the five soil types investigated.

The average inhibitory ability of the Actinomycete populations increased as the pH of the soil samples increased up to 7.5, above which there was a slight decrease. The pH did not affect the size of the Actinomycete population.

The amount of rainfall during the 2 weeks prior to sampling influenced the size of the Actinomycete population but not its antibiotic activity.

The number of Actinomycetes in contact with the roots was considerably greater than in the surrounding soil.

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HETEROSPORIUM DISEASE OF NASTURTIUM AND ITS CONTROL

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(Accepted for publication December 7, 1949)

During the last decade in California a serious leaf-spot disease caused by *Heterosporium tropaeoli* Bond has contributed to a decrease in yield per acre of nasturtium seed (*Tropaeolum majus* L.). At least 100 tons of these seeds for the garden trade in the United States and abroad are produced annually on about 500 acres of the cool coastal area of the State.

The leaf spot was first observed by us in Monterey and Santa Barbara counties, and by C. M. Tompkins in Santa Cruz County, California, during July and August, 1940.[1] Seedsmen stated that the disease had been present for some years in their fields. From the economic viewpoint, this is primarily a disease affecting seed production, since nasturtium is not grown commercially for other purposes. The Heterosporium disease is, however, often a serious problem in garden plantings. It has been seen annually for the last 10 years in coastal gardens from San Diego to San Francisco, and is often quite disfiguring after midsummer.

This paper presents observations, mycological studies, and experiments on the disease conducted in California since 1940, and expands a previous abstract (2).

GEOGRAPHICAL DISTRIBUTION

The fungus has a limited known distribution outside of California. It was collected by H. H. Whetzel at Ithaca, New York, in September 1941.[1] A *Heterosporium* sp., later determined by us to be *H. tropaeoli*, was collected on *Tropaeolum* at Chimaltenango, Guatemala, in June 1942 by A. S. Muller (Muller No. 121; Cornell University, Department of Plant Pathology, Herbarium No. 36378). The leaf symptoms of both of these collections were typical for the disease.

The fungus, which produced a destructive leaf spot of garden nasturtium in Ceylon, was observed and collected by Bond[2] in March 1944 in two localities at 4000–6000 feet elevation. No inoculation studies were reported. He described the fungus and named it *Heterosporium tropaeoli* Bond (4). On the basis of his description and illustration, we consider it to be the same as the California fungus. Wallace and Wallace (13) observed a *Heterosporium* sp. on nasturtium in four localities of Tanganyika Territory in 1947. Because of the widespread destructiveness of the disease it was thought[3] to have been present for some time. Specimens sent to Dr. Bond were identified as *H. tropaeoli* (14), and similar material examined by us was found to be the same as the California fungus. This pathogen has also been collected in

[1] Exsiccati in the Herbarium, Division of Plant Pathology, University of California, Los Angeles.

[2] Letter of August 4, 1948, from Dr. T. E. T. Bond.

[3] Letters of July 20 and August 16, 1948, from Dr. G. B. Wallace.

New South Wales (specimen in the Herbarium, Commonwealth Mycological Institute, Kew).[4]

The genus *Tropaeolum* includes about 50 species native to South America, and collections from that continent would be of particular interest in determining the origin of *Heterosporium tropaeoli*.

HOSTS

No evidence has been found of infection by *Heterosporium tropaeoli* on any genus other than *Tropaeolum*. Observations were made during several years in California trial-ground plantings of seed growers in which there were hundreds of species in small adjacent plots. Infections were severe on nasturtium but occurred on no other plants. There was no significant evidence of differences in varietal susceptibility within the species, *Tropaeolum majus*. However, *T. scemanni* Buch. and *T. peregrinum* L. (canary-bird flower), observed during a single season, were moderately resistant.

SYMPTOMS

The disease occurs on leaf, stem, and seed, but the most characteristic symptom is the leaf spot. The nasturtium seed crop is severely and conspicuously blighted after midsummer, large areas or whole fields having bleached or yellow leaves. Flower production is reduced in quantity and ceases prematurely. This effect, and the failure of the seed to attain normal size because of reduced photosynthetic area, decreases the number and weight of seeds produced per acre.

Leaf spots appear first as tiny red flecks which enlarge to round or angular spots 10–25 mm. in diameter. Such spots are amphigenous, may occur over the leaf blade, and frequently coalesce. Others may start at hydathodes on the margin and become semicircular. The lesions on yellow-flowered varieties are tan without a dark margin (Fig. 1, A), but those on red varieties have brown centers and dark borders (Fig. 1, B). Under moist conditions copious dark greenish-brown conidiophores and spores appear predominantly on the lower surface but to some extent on the upper (Fig. 1, B). Yellowing develops around the spot and may involve the whole leaf blade, causing it to wither. Centers of old spots dry and sometimes crack (Fig. 1, B).

Stem cankers are rarely seen in the field, but they occur very commonly on plants grown from infected seed under moist conditions in the greenhouse. These lesions initiate underground at the point of attachment of the seed to the stem in the hypogeal germination (Fig. 1, C). The stem develops a reddish-brown decay which may progress downward into the roots and, under moist conditions, upward to a point 1 in. above soil level. Soil moisture and inoculum potential of the seed seem to determine the rate of progress of this phase. Seedlings may be girdled and die in the first- or second-leaf stage, or death may not occur until the plant is 5–6 in. high. Infected

[4] Letter of June 20, 1949, from Dr. S. P. Wiltshire.

seedlings turn yellow, die, fall over, and under moist conditions develop profuse dark sporulation. If the fungus develops slowly, roots may form on the stem above the lesion (Fig. 1, D).

Mature yellow fruits from an infected field commonly show irregular discolored areas in the pericarp (Fig. 2, E) produced by masses of dark mycelium that may give rise to abundant conidiophores when held in a moist chamber. No infections of green fruits have been observed, but in mature fruits the mycelium may penetrate the interior of the seed. Saprophytic Alternarias sometimes also cause discolored areas on mature fruits that might be mistaken for those produced by Heterosporium.

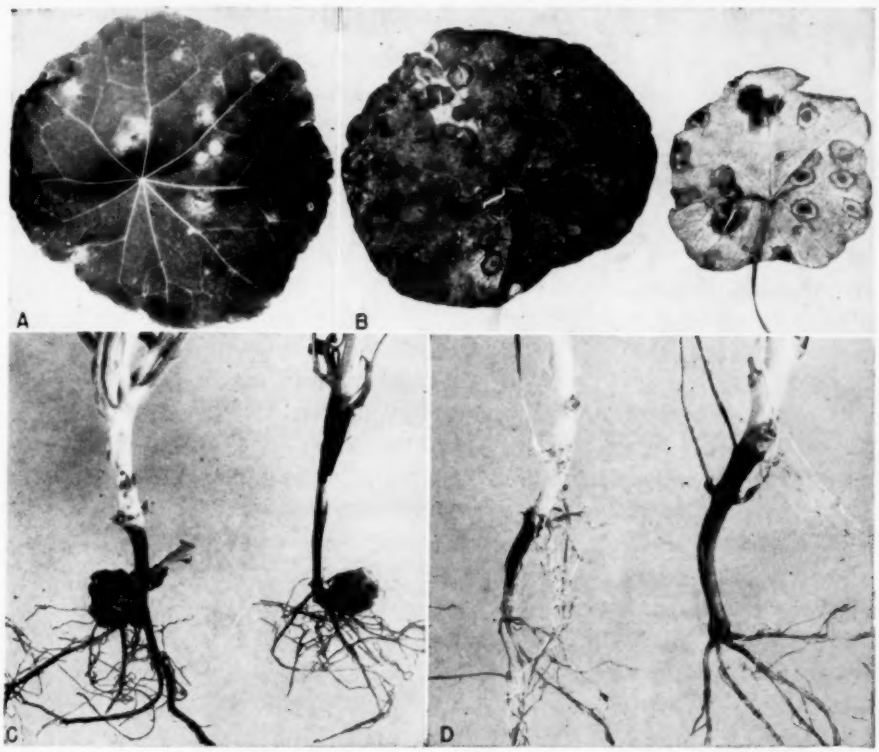

FIG. 1. Heterosporium disease of nasturtium. A. Leaf spots on light-yellow variety. B. Leaf spots on red variety; upper and lower surfaces, latter showing dense sporulation in centers of some spots. C. Stem cankers, showing their origin from old seed, and sloughing of decayed cortex. D. Stem cankers, showing development of adventitious roots above decayed areas.

Other leaf-spotting organisms on nasturtium. There are few other pathogens that cause leaf spots of nasturtium in the United States, and these occur rarely and in localized areas. *Pleospora tropaeoli* Halst. and its Alternaria stage were said (8) to occur on extensive yellow areas of the leaf that later turned brown and became covered with dark spores. Apparently pathogenicity was not demonstrated, and the fungus may well have been the saprophytic *Pleospora herbarum* (Pers. ex Fr.) Rabh. (15). *Cercospora tropaeoli*

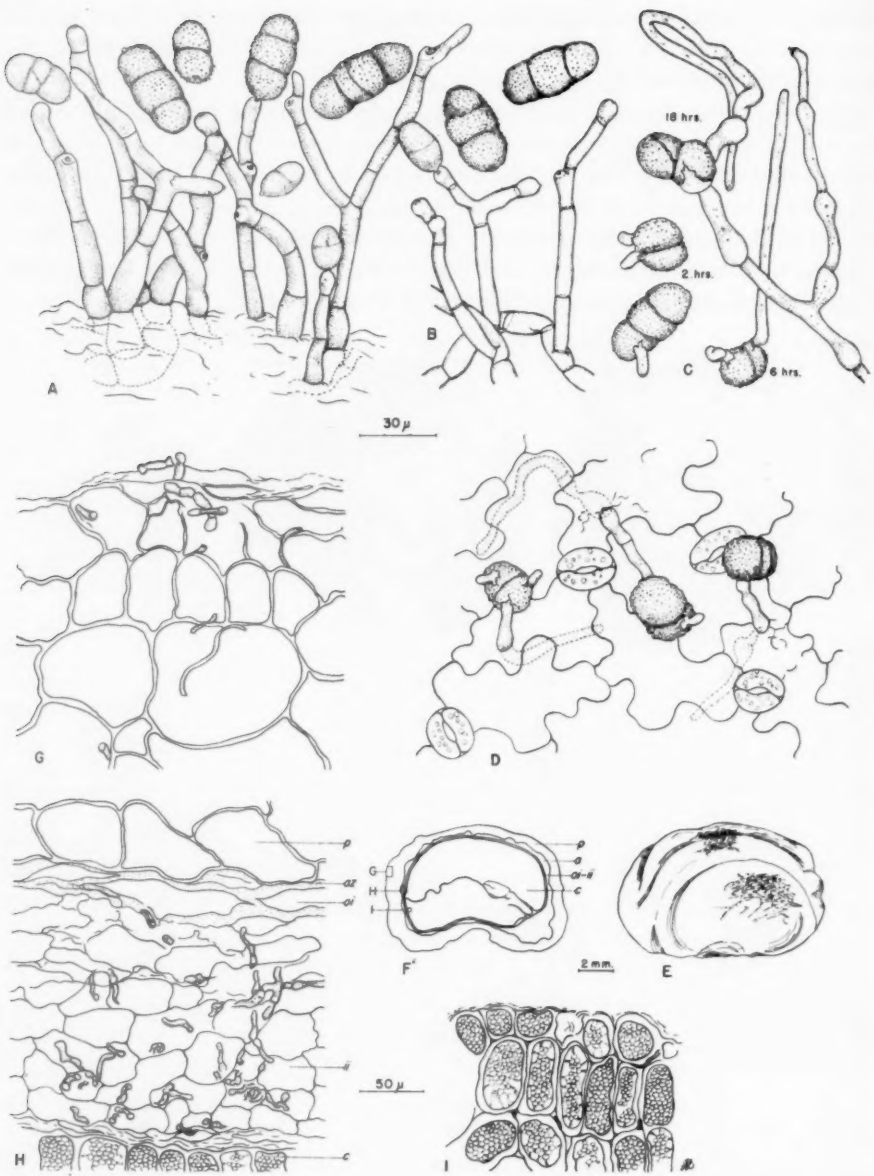

FIG. 2. *Heterosporium tropaeoli* on nasturtium. A. Conidiophores and conidia produced on lower leaf surface, showing typical single structures and the tendency toward fasciculation (at left). B. Conidiophores and conidia produced on water agar plus cold-sterilized natural media. C. Conidia germinating on water agar, after 2–18 hr. D. Conidia germinating on lower leaf surface showing direct penetration, after 17 hr. E. External view of fruit showing dark mycelial masses in the pericarp. F. Section of fruit showing location of enlargements G, H, and I. a = air space; c = cotyledon; ii = inner integument; oi = outer integument; p = pericarp. G. Section of pericarp showing mycelium. H. Section showing infected tissues between inner pericarp and cotyledon. az = zone in which air space develops between pericarp and outer integument. I. Section of infected cotyledon. Camera lucida drawings at three magnifications: A–D comprise one group, E–F another, and G–I the last.

Atk. caused small light-brown spots in nasturtium leaves in Alabama (1), and has been subsequently observed in Brazil and Virginia.[5] A bacterial leaf spot caused by *Pseudomonas aptata* (Brown and Jam.) Stev. was described in 1913 (5) but has since been found only a few times. The spots are at first water-soaked, becoming light brown; they are distinguished from the above and the Heterosporium leaf spots by the absence of dark sporulation.

THE PATHOGEN

The California specimens of *Heterosporium tropaeoli* Bond show some morphological differences from the original description (4), but these are regarded as unimportant because of the great variability of the fungus under different environmental conditions.

Olive brown conidiophores occur singly or rarely in groups of two or three (Fig. 2, A), thus differing from characteristically fasciculate Heterosporiums described by Jacques (10). The conidiophores vary considerably (39–150 × 4–7.5 μ) attaining greatest size under moist conditions. This may account for the greater maxima (175 × 8 μ) in Ceylon. Branching, which is uncommon in California and Ceylon specimens as well as in culture, develops near the point of attachment of a conidium and may be at the lowest scar or higher on the conidiophore. Conidiophores are swollen at the tips and distinctly geniculate, with prominent scars. They develop between epidermal cells and through stomata.

Conidial walls vary from the thickly and minutely echinulate condition described by Bond (4) to verrucose with occasional coarse spines (Fig. 2, A, B). The smaller conidia tend to be echinulate while the larger and the mature dried ones are verrucose. The conidia are olive brown, usually 3-celled but vary from 2 to 4, rarely muriform, cylindrical, slightly constricted at the septa with a broadened basal cell showing a conspicuous scar. They are borne singly instead of in the chains characteristic of other Heterosporiums (Fig. 2, A, B). The 2-celled conidia are 17–35 × 10–21 μ. In some collections most of the conidia were 2-celled, darker brown, thicker walled and more echinulate than in others. The 3-celled conidia are 24–43 × 13–20 μ; the Ceylon spores (4) of this type were 30–40 × 16–22 μ. Four-celled conidia are 38–59 × 15–20 μ. Spores produced on potato-dextrose agar cultures were mostly 2- and 3-celled, the latter measuring 27–41 × 17–21 μ.

Bits of infected tissue on potato-dextrose agar give rise to grayish-white cottony colonies in 3 days; these become olivaceous green and form spores in 5 days at laboratory temperatures. A purple pigment occurs frequently in the growing tips of the mycelium and diffuses into the agar. Hyaline crystals are present in the medium. A stroma (10) consisting of thickened, dark brown, rounded cells is formed, and scattered through it are pseudoparenchymatous clumps suggestive of perithecial initials. However, no perithecia were observed. Cracks form in the agar from the arching of the stroma.

[5] Letter from Dr. C. Chupp, June 24, 1949.

On autoclaved seeds and stems, the fungus develops rapidly, sporulating copiously in 3 days in the laboratory. Sporulation ceases when held at 5° C. and is resumed on return to room temperature.

Monoconidial cultures from the host are of Hansen's (9) M (mycelial) and C (conidial) types. The former produces a dark, olive-green, dome-shaped colony with grayish-white, flexuous aerial hyphae. Conidia are scattered and appear tardily. Type C colonies are appressed, dark olive green, and sporulate copiously. These correspond to types A and B respectively observed by Burt (6) on *Heterosporium echinulatum*.

Extensive field search over several seasons has failed to reveal any evidence of the perfect stage of the fungus, nor has any been formed in culture. Cooke and Harkness (7) described *Sphaeria sarmenti* Cke. and Hark. on stems of *Tropaeolum minus* at San Rafael, California, in December, 1884. They did not mention any conidial stage. The only known specimen of this, in the Herbarium, Royal Botanic Gardens, Kew (Harkness No. 1957), was kindly examined by Dr. G. R. Bisby. According to correspondence in June 1949 with Dr. S. P. Wiltshire, no *Heterosporium* was found, only *Cladosporium* and *Alternaria*. The fungus was later transferred to *Didymosphaeria* as *D. sarmenti* (Cke. and Hark.) Berl. and Vogl. (3). Until the perfect stage of *Heterosporium tropaeoli* is found, any possible relationship to this old collection is undeterminable.

LIFE HISTORY

Infection. Pathogenicity of the fungus was demonstrated (2) by atomizing a suspension of spores from a pure culture onto nasturtium plants. These were held under warm humid conditions for 2 days and returned to the greenhouse. Within a week typical leaf spots developed on inoculated plants, whereas checks remained healthy. Sporulation did not occur until plants were returned to moist conditions. The original fungus was reisolated from these spots. Wallace and Wallace (13) also obtained the fungus in pure culture and demonstrated its pathogenicity by inoculation.

To determine whether infection was aided by injuries, leaves were touched with a hot needle to produce necrotic areas, or scratched with carborundum powder prior to atomizing with spores. Other leaves that had insect-feeding injuries or detectable hydathode abnormalities, and some with no evident damage, were also inoculated. Noninoculated leaves of each of these types serving as checks remained uninfected. Closed cellophane bags were kept over these leaves in the greenhouse for a week. Typical leaf spots developed in all inoculated plants except those with carborundum abrasions which showed generalized invasion. This widespread injury to the surface greatly increased the number of infections. Other injured leaves had as many infections in normal tissue as at points of damage. These results show that infection can occur through injuries and necrotic areas as well as normal tissue.

Conidia from young cultures germinate in water or on water agar in

about 2 hr. Old or dry spores germinate more slowly, conspicuously crack the epispore, and sometimes form racket mycelia (Fig. 2, C).

Laboratory experiments revealed the manner of penetration of uninjured leaves by germinating conidia. The time and method of penetration was determined by removing epidermal strips from these leaves at intervals, and staining with lactophenol cotton blue. The leaves were placed in Petri dishes on moist filter paper and either atomized with a spore suspension or dusted with dry conidia. The germ tubes in the moister plates attained greater length (up to 60 μ) and required more time before penetration than did those (up to 25 μ) in the drier plates. Infections occurred directly through the cuticle of both upper and lower surfaces but not through stomata under these conditions. No definite appressorium was formed, the mycelium merely enlarging slightly with a constriction at the point of entry (Fig. 2, D). No penetration was observed in less than 17 hr. at laboratory temperatures. In 42 hr. abundant subcuticular hyphae had developed and host cells had a reddish discoloration. Such tiny red specks are the first visible symptom of infection. Sporulation was present on the mature spots after the fourth day.

Green fruits could not be infected in repeated trials even when held under moist conditions following mild wounding. However, after these same fruits became yellow during maturation, the fungus developed vigorously in the pericarps, producing conspicuous darkened areas (Fig. 2, E).

In the field the long slender peduncles droop, frequently allowing the maturing fruits to rest on the soil. Because the seed crop requires uniform moisture, it is grown on moderately heavy soil and is ditch-irrigated. The resulting conditions are favorable for infection of maturing fruits. Infection sometimes occurs through the senescent pistil of green fruit, the incipient lesion produced enlarging rapidly as the fruit matures.

The nasturtium seed crop is commonly cut while the plants are still green and only the oldest fruits are mature. These plants are piled on canvas spread on the ground. The high moisture content of the plants enables fruit development to continue for at least a week, and the high humidity favors copious sporulation of the pathogen and infection of the maturing pericarps.

The evidence suggests that *Heterosporium tropaeoli* is a facultative parasite, and not particularly aggressive. It has never been observed on petioles or peduncles, and is able to infect seeds only after maturation and senescence of the pericarp has begun. Infection of stems initiates only at the point of attachment of the old seed remnants (Fig. 1, C, D). It is undetermined why this weak parasite is able so successfully to infect healthy green leaves.

Seed[6] *transmission.* Observations of discolored seeds in commercial lots suggested that they might be a means of disseminating the fungus. Such seeds planted on moist, sterile, black peat in Petri dishes developed copious sporulation of *Heterosporium* on the pericarps. Seeds planted in pasteur-

[6] Botanically this structure is a fruit; the nasturtium "seed" of commerce usually retains the dead pericarp. In this paper, dry fruits are referred to as seed.

ized soil in flats produced seedlings with typical stem lesions (Fig. 1, C, D), whereas those from uninfected or hot-water-treated seed had no symptoms. This is a convenient and reliable method of indexing seed lots to determine the amount of infection, and the results presented here were obtained in this way. Up to 93.2 per cent of seed in commercial lots was found to be infected with *Heterosporium tropaeoli*.

Three-year-old seeds with discolored pericarps planted in pasteurized soil gave rise to seedlings with typical stem lesions. The fungus survives as thick-walled dormant mycelium in pericarp and seed (Fig. 2, E–I). Dry spores lodging on seed surfaces during harvesting probably survive a year or more.

Stained microtome sections showed that the Heterosporium mycelium grows on the surface and through the pericarp (Fig. 2, G) and may extend into the integuments of the seed (Fig. 2, H), sometimes invading the cotyledons (Fig. 2, I). Details of the anatomy of nasturtium seed are given by Kayser (11, pp. 125–141, t. VII).

Primary inoculum. Under California field conditions the primary inoculum arises from sporulation on: (a) stem lesions of plants from infected seed sown in the field; (b) volunteer plants from infected seed in adjacent fields or gardens; (c) overwintering infected plants in near-by gardens or wasteland.

The reddish-brown stem lesions extend above the soil level causing the seedlings to fall over. Because the plants are then in contact with moist soil, the fungus rapidly invades the tissue and sporulates profusely. These spores are blown to leaves of other nasturtiums and supply a source of inoculum within the field.

Seeds left in the field following harvesting operations in California start to grow with the advent of winter rains. Some of these volunteer plants are infected and supply a second initial source of inoculum until the old fields are plowed and prepared for planting. A rotation of 1–4 years between nasturtium crops in a given field is an established practice to reduce varietal mixing. Therefore, no residual seed is present in a field when replanted, and the fungus is introduced with seed or by air-borne spores.

Nasturtium plants frequently survive the winter in protected locations in coastal California. Therefore, "escapes" starting from rubbish piles in uncultivated areas continue growth for several years, perpetuating the pathogen. Nasturtiums in home yards near commercial plantings act as further reservoirs of fungus inoculum. Such areas have often been observed to supply the air-borne spores that initiate infection in adjacent commercial nasturtium fields planted with treated seed.

In the experiments in which hot-water-treated seed was planted in fields where nasturtiums had been grown several years previously, there was no evidence of survival of the pathogen in soil. Because of this fact and the nature of the fungus, soil carry-over is considered to be improbable. The

refuse from nasturtium fields decays promptly, and might sustain the fungus over one season, but not longer.

Secondary spread. The leaf spots produced from the primary inoculum develop rather slowly under California conditions, the disease ordinarily becoming severe when fields reach full bloom after early July. Copious sporulation occurs on the undersurfaces of the leaf spots (Fig. 1, B) and the conidia are air-borne. Since the fungus sporulates on spots one week after infection, the biotic potential is enormous, once environmental conditions become favorable.

ENVIRONMENTAL RELATIONS

Field observations suggest that temperatures of 18°–29° C. are favorable for the pathogen, but no detailed studies have been made. While the fungus is present in spots on living leaves during practically every month in coastal California, it generally reaches epidemic proportions in July, when the temperatures also are highest in the seed areas. Limited tests in the laboratory showed that spore germination and penetration of the leaf were greatest at about 25° C.

Moderately high humidity is required for sporulation on stem lesions and leaf spots. Wet soil conditions favor the stem-rot phase of the disease. During the months of April to September when seed nasturtiums are grown, there is almost no rain in the coastal area. Because nasturtium leaves are extremely difficult to wet, drops from irrigation sprinklers or rain roll off and probably play little part in favoring infection. However, dew and fog, which are fairly common during the summer months, condense on leaves, permitting infection to occur. The fungus probably also infects through guttation drops present at hydathodes during humid nights.

CONTROL IN SEED FIELDS[7]

A consideration of the nature of the crop, its culture in seed fields, and the character of the fungus caused us to first explore the possibilities of eradicatory treatments rather than field spray applications. The apparent limitation of the fungus to a single host and the probability that the pathogen is not a soil organism suggested that a successful eradicatory seed treatment could break its cycle. Because of large areas of land available for seed fields, effective isolation from other nasturtiums seemed possible. The lower cost and greater convenience of seed treatment as compared with a spray program was another attractive feature. Due to the success attained by hot-water treatment of the seed, no spraying experiments have been necessary.

Seed treatment. In early tests, chemical seed treatments (New Improved Ceresan, Semesan, and red cuprous oxide) failed to prevent development of stem lesions from infected seed. After internal transmission of the fun-

[7] The excellent cooperation in this portion of the work by Mr. Warren F. Locke of the Waller-Franklin Seed Co., Guadalupe, Mr. Marshall Lewis of the Denholm Seed Co., Lompoc, and Mr. Ian Sinclair of the Bodger Seed Co., Lompoc, California, is acknowledged with pleasure.

gus was demonstrated, this type of treatment was abandoned in favor of hot water. Preliminary tests with hot water showed that immersion at 51.7° C. for 30 min. killed the fungus without injuring the seed, and that 53.8° C. for the same period decreased germination.

The treatment finally adopted has been used commercially for the past 6 years and has now restored seed yields to the levels preceding disease epidemics. Seed is soaked in cool tap water in large galvanized cans for 1 hr. in order to displace the air between the loose pericarp and the seed (Fig. 2, F). Weighted screens are used to prevent floating. As there is considerable swelling of the seed, the cans should not be filled more than two-thirds full. The drained seed is enclosed in wire screen boxes that are then plunged into a large tank of circulating water held at 51.7° C. (125° F.) and are frequently turned over during the 30-min. treatment. The containers are then removed and immediately cooled by flooding with tap water. The seed is spread out in thin layers on screens to dry either outdoors or in a heated room or dehydrator. Drying should be accomplished in 12–20 hr. without overheating.

During the experimental work, we treated about a ton of nasturtium seed over a 4-year period, and commercial seed companies have treated at least 6 tons in the last 5 years. Representative experimental data on the results of the treatment and its effect on germination are presented in table 1.

There was an average germination reduction of 2.9 per cent, ranging from a reduction of 29.0 per cent in 3-year-old seed to an apparent increase of 12.8 per cent in one lot. A commercial seed company treated 1650 pounds of seed of 29 varieties or lots in 1946 and found an average germination reduction of 6.3 per cent, with a maximum loss of 32.5 per cent in one lot. It is evident that there is negligible injury to seed from the treatment.

In the 13 representative lots of table 1 in which 0 to 47 per cent of the untreated seed was infected, only a single treated seed was found to carry the fungus, and this probably represented a contamination.

Satisfactory control of the disease has been obtained by this treatment in extensive tests, and the method is now being used commercially by three companies for seed planted in their fields. The leaf-blight phase of the disease was almost completely controlled in plants grown from treated seed under isolated conditions, with development of only a few scattering leaf spots from air-borne inoculum. Even in some inadequately isolated fields the appearance of the disease was so delayed that it did not become a serious problem. With adequate treatment and isolation the seed produced was clean and light colored in contrast to dark seed from infected fields.

Isolation of planting area. The conidia of *Heterosporium* are of the Xerosporae type (12). Since they are developed profusely on infected leaves and are air-borne, isolation of the seed fields is required to control the disease. Field evidence that the conidia are wind-borne is given in table 2.

Of 7 lots of seed treated prior to planting in 1944, 6 were grown in well-isolated areas. When the crops were indexed in 1945 only the nonisolated

TABLE 1.—*Control of Heterosporium disease of nasturtium by hot-water treatment of seed. Planted in pasteurized soil in greenhouse April 12, 1945. Final data taken July 7, 1945*

Variety and lot	Untreated seed[a]		Hot-water treated seed[a]		Percentage germination reduction
	Percentage germination in soil	Percentage viable seed with Heterosporium	Percentage germination in soil	Percentage viable seed with Heterosporium	
1. Tall Single Mixed	79	25.3	75	0.0	5.1
2. Dwarf Single Mixed	66	47.0	69	1.4[b]	+4.5
3. Semidouble Dwarf Golden Globe	77	26.0	.....	.....	.....
4. Semidouble Dwarf Scarlet Globe	54	1.9	51	0.0	5.6
5. Semidouble Gleam Scarlet Gleam	45	0.0	43	0.0	4.4
6. Semidouble Scarlet Gleam	54	0.0	48	0.0	11.1
7. Semidouble Scarlet Gleam	78	3.8	85	0.0	+8.8
8. Semidouble Scarlet Gleam	91	0.0	95	0.0	+4.4
9. Semidouble Golden Gleam	65	12.3	73	0.0	+12.8
10. Semidouble Giant Gleam Sun Gleam	62	4.8	44	0.0	29.0[c]
11. Semidouble Glorious Gleam Hybrids	82	0.0	87	0.0	+6.1
12. Semidouble Glorious Gleam Hybrids	95	2.1	84	0.0	11.6
13. Semidouble Glorious Gleam Hybrids	82	0.0	78	0.0	4.9

[a] Results in each test based on a 100-seed sample.

[b] This single infected seed possibly represents an error in handling or a contamination.

[c] Seed 3 years old at time of treatment.

TABLE 2.—*Increase in seed-borne Heterosporium during 2 years, as influenced by amount of infection in seed planted and by isolation of fields*

Variety and lot	Growing area in 1946	Percentage seeds with Heterosporium. Index of seed grown in year[a]:			Percentage germination (in soil) 1946
		1944	1945	1946	
1. Semidouble Dwarf Gem[b]	1	...	0.0	52.5	80
2. Semidouble Dwarf Scarlet Globe[b]	1	0	.....	76.9	91
3. Semidouble Dwarf Golden Globe[b]	1	0	6.5	93.2	88
4. Tall Single Choice Mixed	2	0	0.0	3.1	96
5. Dwarf Single Choice Mixed	3	...	0.0	1.1	92
6. Semidouble Scarlet Gleam	4	0	0.0	3.4	88
7. Semidouble Glorious Gleam Hybrids	5	0	0.0	6.0	84

[a] The 1944 seed was hot-water treated and therefore free of infection. This was used for planting in 1945. Seed of the 1945 crop was indexed and planted in 1946. Seed of the 1946 crop was indexed. Only the 1944 seed was treated.

[b] Infection in 1946 in area 1 started with variety 3. Variety 1 was grown in a strip parallel to variety 3 and 500 feet from it; variety 2 was 200 feet from variety 3. Strips were parallel to the direction of the prevailing wind.

one was found to have infected seed. These 1945 seed lots were planted without treatment in 5 areas with varying degrees of isolation in 1946. Lots 1–3 showed the rapid increase in disease possible when infected seeds are planted in the same or an adjacent field. Lots 4, 5, and 6 grown in areas 2, 3, and 4, isolated from infected plants but still within the same small coastal valley, had little infection. Lot 7 in area 5 was less well isolated, and infection increased to 6.0 per cent.

Since this disease is commercially important only in seed-growing areas, the emphasis in control in California has been on preventing serious loss rather than eliminating the disease. However, one company that used this treatment stated that their fields were free of Heterosporium infection in 1948 and 1949. On the basis of the above data and practical experience, it is considered safe to treat seed stock in alternate years, provided it is not planted closer than a half mile (preferably a mile) to any extensive source of inoculum. It is unsafe, however, to delay treatment through the third season. Attention should be given to adjacent sources of inoculum, particularly those in the direction of the prevailing wind. Rotation periods that eliminate volunteer nasturtiums within the field are very important.

Dry-land cultivation. Because of the moisture and moderately warm temperature required for development of this disease, one company tried advancing the planting date in Ventura County so that the crop was grown during the cool winter months and harvested in May. Since nasturtiums are sensitive to low temperature, this is possible only in frost-free areas. Because of occasional seasonal rains, no irrigation is necessary and the crop can be produced at this season on hilly land. The method has given good results, but requires further testing before it can be recommended.

Indexing of seed lots. A method was devised for determining the percentage of infected seeds in a given lot and for indicating the need and effectiveness of treatment. Soil is pasteurized in flats, each of which is then planted with 100 seeds of the lot to be indexed. The flats are held in the greenhouse and frequently watered. Dying of small seedlings may occur in 2–3 weeks, and in 3–5 weeks stem lesions begin to appear on larger plants. This method does not indicate the number of infected dead seeds. Total number of infected seeds, germinable or dead, can be determined by placing them on sterile, moist, black peat held under varying temperatures, and observing for Heterosporium sporulation on the pericarps.

CONTROL IN GARDENS

Nasturtiums survive the winter in many areas of California and practically always volunteer the next season, making control in gardens difficult. This carry-over can be prevented by elimination of the plant for one year. Subsequently only treated seed should be planted. The diplomatic problem of control of the disease in the neighbor's yard should not be overlooked.

SUMMARY

A disease caused by *Heterosporium tropaeoli* is important after midsummer on nasturtiums grown for seed and as a garden ornamental in coastal California. The disease has been reported also in New York, Guatemala, Ceylon, New South Wales, and Tanganyika Territory. The leaf spot phase often causes blighting and premature ripening of entire fields. Basal stem lesions and discolored seeds are also produced. Seed is reduced in quantity and quality.

The fungus is internally and externally borne in up to 93.2 per cent of commercial "seed," surviving at least 3 years as thick-walled mycelia in pericarp and seed. Green fruits remain uninfected, but following maturation of the pericarp, infection occurs under the favorable moist conditions beneath the plant. Seedlings from infected seeds develop basal stem lesions on which sporulation occurs.

Primary inoculum arises from sporulation on: (a) stem lesions of plants from infected seed sown in the fields; (b) volunteer plants from infected seed in adjacent fields or gardens; (c) overwintering infected plants in near-by gardens or wasteland.

The disease can be controlled by the following hot-water seed treatment: presoak for 1 hr. in cool water; treat in water at 51.7° C. (125° F.) for 30 min.; cool at once; dry within 12–20 hr. There was negligible germination reduction (2.9–6.3 per cent) in new seed, but old seed lots sustained heavier losses (up to 32.5 per cent). The pathogen was eliminated from treated seed. The treatment has been experimentally and commercially used over a 6-year period, increasing seed yield and quality.

Infection can be minimized by planting treated seed in fields at least a half-mile away from any inoculum source. With such isolation, seed treatment in alternate years is sufficient. Even though fields are nearer the inoculum source a satisfactory yield can be obtained by treatment every year.

Because of successful field control of this disease by seed treatment, no spray applications have been necessary.

Seeds may be easily indexed for infection by growing for 5–6 weeks in flats of pasteurized soil and observing for seedling stem lesions.

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SODIUM PENTACHLOROPHENATE AND OTHER MATERIALS AS ERADICATIVE FUNGICIDES AGAINST SCLEROTINIA LAXA

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INTRODUCTION

According to earlier studies (7), an aqueous solution of sodium pentachlorophenate applied as a spray to dormant apricot and almond trees destroyed many of the conidial tufts (sporodochia) produced by *Sclerotinia laxa* Ader. and Ruh. on twigs blighted the previous spring. This material did not, however, destroy the mycelium of *S. laxa* inside the twigs. Consequently, when this treatment was given several weeks before the trees blossomed, a supply of inoculum reappeared, under some conditions, before the susceptible (blossoming) period of the host came to an end. When the treatment was given at the time the unopened flowers emerged from the winter buds, many of the existing conidia were destroyed and only a few new ones were produced before the end of the blossoming period (8). Because of excessive blossom and twig injury it was not feasible to apply sodium pentachlorophenate after the buds began to open.

Through necessity, therefore, the eradicative treatment in subsequent trials was given before the scales of the winter buds separated. Under such circumstances the regeneration of new conidia on treated twigs became an important consideration. This phenomenon was followed in the field and attempts were made to determine the factors influencing it. Also considered was the possibility of strengthening the standard protective spray program with an eradicative treatment of sodium pentachlorophenate.

In the course of these studies, a number of chemical compounds were tested for their eradicative action against *Sclerotinia laxa*. The results of these tests are summarized briefly herein.

GENERAL PROBLEMS IN THE EVALUATION OF ERADICATIVE TREATMENTS

The function of an eradicative treatment in preventing blossom infection by *Sclerotinia laxa* has been discussed elsewhere (6). Monocalcium arsenite is found to be effective against a number of fungi (5), and results obtained with this material on apricots and almonds (6) and on sour cherries (1) prove that brown-rot blossom blight can be materially reduced if a major portion of the conidia is eliminated from the tree. Presumably, therefore, conidia produced on diseased twigs and flowers, and on brown-rot-infected fruit which remain on the tree, are the only important means whereby *S. laxa* propagates itself in nature. On almond the conidia develop largely on affected twigs and the blossoms that are attached to such twigs, fruit infection being rare. In late winter the fungus produces numerous sporodochia

on twigs blighted the previous spring. Hereafter such twigs are called "source twigs."

The total quantity of conidia available for infection, designated the inoculum level, depends on (a) the number of source twigs, (b) the proportion of source twigs producing conidia, (c) the quantity of conidia on those source twigs producing them, and (d) the proportion of conidia germinable at the time infection occurs. Inoculum level, therefore, is one of the elements of inoculum potential, which is regarded (4) as the equilibrium between the amount of fungus material available, number of hosts, distance between hosts, randomness of host distribution, and weather factors.

Since the number of source twigs in badly affected trees sometimes runs into thousands, a count of such twigs is not feasible and, if made, probably would be subject to large errors. Consequently, a determination of the inoculum level was not attempted in this work. Instead, the sporodochial index employed in earlier work (6) was the measure of the response of the fungus to the fungicide. The sporodochial index, being the average number of sporodochia per conidium-producing source twig, expresses the intensity of conidium production. It is a satisfactory criterion for determining the effect of the fungicide on the fungus but is not necessarily proportional to the amount of inoculum available for infection (6, p. 572). For this reason the incidence of blossom infection will not necessarily vary with the sporodochial index, although, in some data presented later, these two elements have a considerable degree of correlation.

The eradicative action of a fungicide employed against *Sclerotinia laxa* is exerted in either or both of two ways, depending on the time of treatment relative to sporodochial production. First, the conidia present at the time of treatment may be destroyed. Second, further conidial production may be prevented. Destruction of inoculum refers to the first, and repression of inoculum refers to the second. Inasmuch as prevention of sporodochium production is rarely complete, repression is a more exact description of this phenomenon than is suppression.

FIELD TRIALS OF SODIUM PENTACHLOROPHENATE

Methods and procedures. Conidia of *Sclerotinia laxa* are readily disseminated by wind (10). In orchard trials of sodium pentachlorophenate, therefore, it was necessary to guard against infection from conidia brought into the treated plots by air currents. Two procedures were adopted for this purpose: (a) The eradicative material was applied to square plots containing 100 or more trees, and records on blossom infections were taken only on trees at the center of each plot. (b) Inasmuch as southerly winds prevailed in this locality, the eradicative treatment was given to 9-tree plots distributed along the south side of an orchard adjacent to an open field and thus located to windward of untreated trees. Although it is probable that neither of these procedures avoids all blossom infection by conidia produced outside the treated plot, judging from a study (10) of the spread of brown-

rot blossom blight in apricot orchards, either should lessen the chance for this type of infection.

The sodium pentachlorophenate preparation was applied to almond trees a few weeks before the blossom buds began to open. Over a period of years the time of applying the spray varied between January 7 and 22.

When protective sprays were included in the program, these were applied during the "popcorn stage" or after the unopened blossoms had emerged from between the winter bud scales. The popcorn stage usually occurs sometime during the first half of February.

At the time the trees were in full bloom, 100 source twigs from each plot were examined and the sporodochial indices determined. In determining these values for source twigs which had received the eradicative spray it was necessary to exercise discretion in recording which sporodochia were and which were not injured by the treatment. The degree of injury of these structures by sodium pentachlorophenate varied according to conditions. Many were so badly injured as to be unrecognizable except by microscopic means and these, of course, were automatically excluded from counts. Others were discolored only on the surface and not in the center of the conidial masses; in these, microscopic examinations revealed that the conidia on the ends of the closely packed chains were shriveled and nonviable, whereas those at the base of each chain were uninjured. On the whole, discoloration and shrinking of the entire sporodochium indicated injury to most of the conidia thereon. For purposes of record, therefore, the sporodochium was considered destroyed only if it was completely discolored and noticeably shrunken.

The foregoing method of determining the efficacy of the eradicative treatment did not, of course, differentiate between destruction of inoculum and repression of inoculum development. Under some conditions, as will be noted later, sodium pentachlorophenate prevented the development of sporodochia, and also destroyed those present when the spray was applied (7).

To determine the effect of treatment on the disease, 500 blossom-producing spurs and terminal shoots were examined in each plot a few weeks after the end of the blossoming period. Ordinarily, by this time, spurs and terminal shoots that bore infected blossoms had withered and were easily recognized.

Effect of sodium pentachlorophenate on the amount of conidia produced on source twigs. Tables 1 and 2 contain results of tests conducted in the manner described for avoiding blossom infection from conidia produced outside of the treated plots. In columns 2, 3, and 4 of table 1, and column 4 of table 2, are data on the number of sporodochia per source twig. Wherever possible the sporodochial indices for nontreatment were obtained from trees receiving no fungicide whatever. In some cases, however, it was necessary to employ trees which had received a protective spray, usually Bordeaux mixture, at the blossom-emergence stage. According to results in

table 1, Bordeaux had little or no effect on the number of sporodochia. Calavan and Keitt (1) also concluded that Bordeaux did not affect sporodochial development.

Tests reported in table 1 were conducted for 3 years on duplicate 9- to 12-tree plots situated along the south side of an orchard adjacent to an open field. Column 5 of this table gives the 3-year averages for sporodochial indices. Judging from the values at the bottom of column 5, significant differences at the 5 per cent statistical level existed between sporodochial indices for nontreatment and those for the sodium pentachlorophenate treatments.

TABLE 1.—*Comparison of sodium pentachlorophenate as an eradicative fungicide and Bordeaux as a protective fungicide for controlling brown-rot blossom blight in almonds, Sacramento County, California*

Treatments,[a] materials in pounds per 100 gal.	Number of sporodochia per source twig				Percentage of blossoming twigs blighted			
	1944	1945	1946	Av.	1944	1945	1946	Av.
Unsprayed	2.4	5.8	1.7	3.3	21	22	14	19.0
Sodium pentachlorophenate 4–100	0.7	1.4	0.5	0.9	9	6	5	6.7
Sodium pentachlorophenate 4–100; Bordeaux 10–10–100	0.7	1.1	0.7	0.8	4	1	1	2.0
Bordeaux 10–10–100	2.1	4.6	1.9	2.9	11	8	5	8.0
Sodium pentachlorophenate 4–100, plus Bordeaux 2–2–100; Bordeaux 10–10–100	.....	1.7	.....	.....	.....	3	.....	.....
Calculated *F.* value				6.12[b]				46.6 [c]
Difference for significance, 19: 1 odds				1.82				3.65
99: 1 odds				2.76				5.52

[a] The sodium pentachlorophenate preparations, including that containing Bordeaux, 2–2–100, were applied in the dormant season as follows: 1944, January 13; 1945, January 15; 1946, January 14. The Bordeaux treatment was given after the unopened blossoms emerged from the winter buds as follows: 1944, February 16; 1945, February 12; 1946, February 8. Four ounces of sodium lauryl sulfate per 100 gal. were added to each preparation of sodium pentachlorophenate.

[b] Exceeds tabular value for 19: 1 odds.

[c] Exceeds tabular value for 99: 1 odds.

As stated before, Bordeaux apparently had no effect on sporodochial numbers. Assuming this to be the case for all tests reported in tables 1 and 2, the relation between the intensity of sporodochial development on nontreated twigs (Bordeaux but no sodium pentachlorophenate) and that on treated twigs may be examined in columns 2 and 3 of table 3. The intensity or level of sporodochial development on nontreated twigs varied greatly. The sporodochial indices for treated twigs (Column 3) tend to rise as the nontreated values (Column 2) increase. Though the degree of variance within these two sets of data differs, corresponding values are closely correlated, as indicated by the coefficient (Table 3). Consequently, the ratios in column 4, though somewhat variable, show no consistent tendency to rise or

TABLE 2.—*Effect of sodium pentachlorophenate on the conidial inoculum and brown-rot blossom infection in almonds, 1947*

Orchard and location	Treatment[a]	Stage or date of application[b]	Sporodochia per source twig	Blossoming twigs blighted
			Number	*Pct.*
Roeber (Winters)	Bordeaux, 14–14-100	Pre-blossom	8.7	19
	Eradicant, 8–100; and	Jan. 6		
	Bordeaux 14–14–100	Pre-blossom	2.2	1
House (Escalon)	Bordeaux, 10–10–100	Pre-blossom	9.6	16
	Eradicant, 8–100; and	Jan. 13		
	Bordeaux, 10–10–100	Pre-blossom	4.3	2
Gray (Esparto)	Bordeaux, 10–10–100	Pre-blossom	15.0	41
	Eradicant, 8–100; and	Jan. 18		
	Bordeaux, 10–10–100	Pre-blossom	2.0	8
Little (Empire)	Basic copper sulfate, 5–100	Pre-blossom	19.0	59
	Eradicant, 8–100; and	Jan. 15		
	basic copper sulfate, 5–100	Pre-blossom	8.3	34
Locke (Arbuckle)	Unsprayed[c]	.....	21.4	62
	Eradicant, 8–100	Jan. 22	7.3	28
	Eradicant, 8–100; and	Jan. 22		
	Bordeaux, 10–10–100	Pre-blossom	7.3	7

[a] The eradicant consisted of 47 per cent sodium pentachlorophenate and 53 per cent bentonite with a small amount of wetting agent. The suspension (8 lb. in 100 gal. water) was applied while the trees were dormant, on the dates indicated.

[b] Bordeaux was applied when the unopened blossoms emerged from the winter buds, the exact dates being unknown.

[c] No fungicide.

fall with the intensity of sporodochial development on nontreated twigs. Presumably, therefore, under conditions prevailing during the 4 years of

TABLE 3.—*Relation between the intensity of sporodochial development by Sclerotinia laxa on nontreated and that on sodium pentachlorophenate-treated twigs*

Block	Average number of sporodochia per source twig[a]		Proportion of sporodochia eliminated by treatment ($1-S/U$)
	Nontreated (U)	Sodium pentachlorophenate-treated (S)	
1	2.40	0.70	0.71
2	3.30	0.90	0.73
3	5.80	1.40	0.76
4	8.70	2.20	0.75
5	9.60	4.30	0.55
6	15.00	2.00	0.87
7	19.00	8.30	0.56
8	21.40	7.30	0.66
Average	10.65	3.39	0.69
Calculated F. value for treatment			11.23[b]
Difference for significance 19: 1 odds			4.58[c]
99: 1 odds			6.77[c]
Coefficient of correlation between data in columns U and S			0.87 ± 0.06

[a] Data taken from tables 1 and 2.

[b] Exceeds tabular value for 19: 1 odds.

[c] Pertains to averages at bottom of columns 2 and 3.

tests, the degree of eradicative action was independent of the intensity of inoculum development. A similar relationship was observed with the monocalcium arsenite eradicative treatment (6, Fig. 4, p. 573). There, as might be expected, the degree of eradicative action differed for two concentrations of monocalcium arsenite.

Although some sporodochia usually were present when the eradicative treatment was given, maximum production of these structures usually occurred between the time of treatment and blossoming time, when the final counts were made. Differences in the behavior of the fungus on treated and nontreated twigs were observed during this period. In 1947, at the Locke

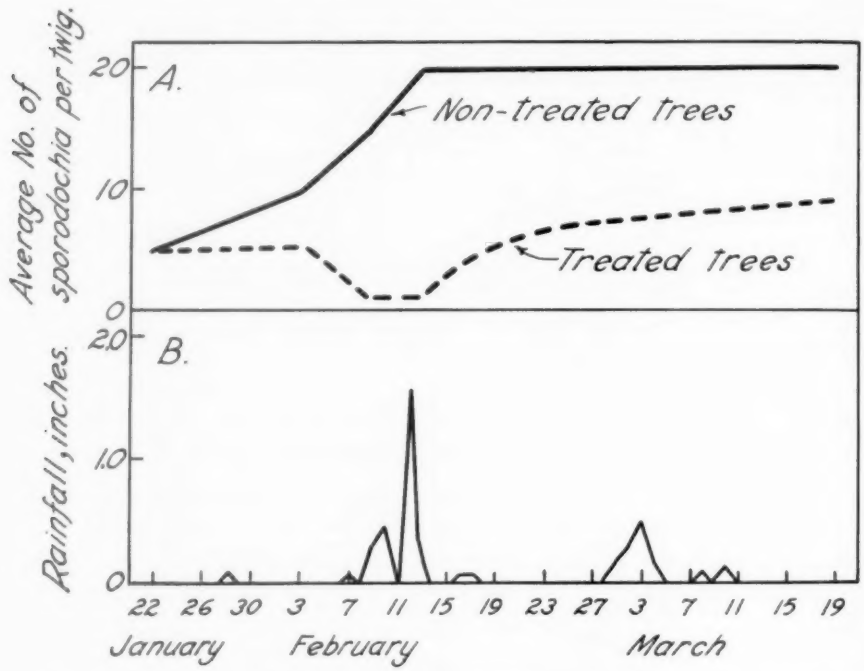

FIG. 1. A. Sporodochial production by *Sclerotinia laxa* on sodium pentachlorophenate-treated and nontreated twigs. Locke orchard, Arbuckle, California. B. Rainfall record, 1947, for Colusa, California, 13 miles northeast of Arbuckle.

orchard, for example, the development of sporodochia on tagged twigs was followed from the day of treatment, January 22, until March 19, a week or more after the blossoming period. Here (Fig. 1, A) the sporodochial index was 5 on the day of spraying. Between January 22 and February 13 the index for nontreated twigs rose to 20 and remained at this level until observations were discontinued. For treated twigs, however, the index remained at 5 until February 3, then declined and remained at the lower level until February 13. After February 13 it steadily increased, attaining a value of about 8 at the blossoming period. Apparently, therefore, between February 3 and 13 the fungicide not only destroyed sporodochia pres-

ent at the time of treatment, but throughout the period from January 22 to February 13 prevented development of these structures at a time which, judging from the data for nontreated twigs, was favorable for sporodochial production. After February 13, however, the fungicide no longer possessed the ability to prevent sporodochial formation.

During periods of rapid sporodochial development, the conidial masses when first appearing were white. The conidia were normal in shape but smaller and lower in germinability than those from the gray, older sporodochia. A week or so was required for these conidial masses to mature. This color difference between young and old sporodochia proved valuable in following conidial development in the field. As we have seen (Fig. 1), in 1947

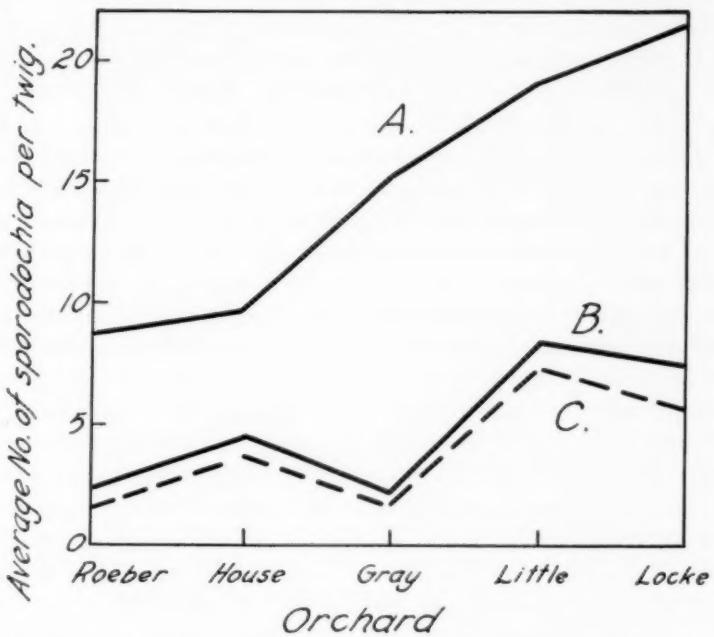

FIG. 2. Production of sporodochia by *Sclerotinia laxa* at the experimental orchards during 1947. A. On trees receiving no eradicative spray. Almost all sporodochia here were gray. B. On trees which received the eradicative spray; including both gray (mature) and white (immature) sporodochia. C. Recently developed (white) sporodochia on trees which received the eradicative spray.

most sporodochia on nontreated trees at the Locke orchard developed in late January and early February. These had attained the gray color of mature sporodochia by blossoming time, which fell between the middle and latter part of February. On the other hand, sporodochia did not develop on treated trees to any extent until after February 13. These were white throughout most of the blossoming period.

Utilizing color as a criterion for approximating the age of the sporodochia, figure 2 was constructed to illustrate the relation between destruction of sporodochia and repression of sporodochial development in the five

experimental orchards during 1947. Curve A represents the intensity of sporodochial development in nontreated trees, and curve B, that in treated trees. From the vertical distance between curves A and B, therefore, one may estimate the relative amount of inoculum eliminated by the combined destructive and repressive action of sodium pentachlorophenate. Curve C gives the average number of white immature sporodochia per treated source twig. Thus, it is seen from the small differences between curves C and B, most of the sporodochia on treated twigs in all five orchards were newly formed. Presumably, therefore, at the Roeber, House, Gray, and Little orchards, as at the Locke orchard, development of the inoculum was delayed by the sodium pentachlorophenate treatment.

The eradicative efficacy of sodium pentachlorophenate was much lower in 1948 than in the preceding four seasons. According to results of tests conducted on Drake almonds near Arbuckle, Colusa County, and Ne Plus Ultra almonds near Esparto, Yolo County, only 37 to 46 per cent of the inoculum was eliminated by the fungicide. Ten days after the spray was applied (January 14 to 16) little or no damage to sporodochia was detected. In fact, it was not until blossoms began to emerge from the winter buds, during the first week of February, that appreciable destruction of sporodochia occurred. Then, as in 1947, eradicative action of the fungicide accompanied a period of rainy weather.

Effect of sodium pentachlorophenate on the incidence of blossom infection. To what degree did the elimination of a considerable proportion of conidial inoculum affect blossom infection? According to the results reported in table 1, the sodium pentachlorophenate treatment alone reduced the incidence of blossom infection 58 per cent in 1944, 73 per cent in 1945, and 64 per cent in 1946; Bordeaux alone reduced infection 48, 64, and 64 per cent in the respective years; whereas sodium pentachlorophenate followed by Bordeaux reduced it 81, 95, and 93 per cent respectively. A weak Bordeaux (2–2–100) added to the sodium pentachlorophenate preparation in one trial perhaps decreased the efficacy of the eradicant treatment slightly.

A number of almond growers applied sodium pentachlorophenate experimentally in 1946, but the results were inconclusive because of sparse blossom infection. In one orchard, however, where the disease developed moderately, the percentage of blossoming twigs blighted was 26 on trees sprayed with Bordeaux 10–10–100 on January 22 (much too early for effective protection), and 11 on trees given the sodium pentachlorophenate treatment.

The season of 1947 was marked by severe blossom infection. As noted earlier, unsprayed trees were not available that year in four of the five tests conducted on a commercial scale. Comparisons were made between plots receiving a protective spray and those receiving both the eradicative and protective sprays. At the Roeber, House, and Gray orchards the percentages of blossoming twigs blighted were 19, 16, and 41, respectively, in plots

receiving only the Bordeaux treatment, and 1, 2, and 8, respectively, in plots receiving both the sodium pentachlorophenate and the Bordeaux treatments. At the Little orchard 59 per cent of blossoming twigs were blighted in trees receiving only a basic copper sulfate treatment and 34 per cent in trees receiving both the sodium pentachlorophenate and basic copper sulfate treatments. At the Locke orchard the results were as follows: no fungicide whatever, 62 per cent blossoming twigs blighted; sodium pentachlorophenate, 28 per cent; and sodium pentachlorophenate followed by Bordeaux, 7 per cent.

The most striking feature of these data is the variability of the disease between plots receiving the protective spray only. For example, between Bordeaux plots at the House and Gray orchards the disease varied more than 100 per cent. Differences in the timing of the protective spray, in thoroughness of coverage, and in local weather conditions may have been responsible in part for such variability. It is noteworthy, however, that the incidence of the disease in the plots receiving the protective sprays rose as the sporodochial indices for these plots increased (Table 2). Thus, control of blossom infection by a protective spray probably was most difficult when the intensity of sporodochial production was greatest.

The low eradicative efficiency of sodium pentachlorophenate in the 1948 season was followed by unsatisfactory control that year. In two tests, blighting of blossoming twigs was reduced only 31 and 41 per cent. Apparently, moreover, results obtained (3) in another locality were not uniformly satisfactory.

In 1949, at Arbuckle, Colusa County, a program consisting of the sodium pentachlorophenate treatment on Jan. 21, followed on March 8 by one of Bordeaux 10–10–100 at the popcorn stage, was compared with programs consisting of one (popcorn) Bordeaux treatment on March 8 and two (popcorn and 50 per cent of blossoms open) Bordeaux treatments on March 8 and March 17. The sodium pentachlorophenate and Bordeaux reduced the incidence of disease 76 per cent; the single Bordeaux reduced it 48 per cent; and the two Bordeaux treatments reduced it 82 per cent.

Almonds blossomed very late in 1949 because of cold weather; consequently, a much longer period than usual elapsed between the time of applying sodium pentachlorophenate and the emergence of blossoms, and the fungus was thus afforded adequate time to produce a new supply of conidia in treated trees. In spite of this, the sodium pentachlorophenate aided materially in controlling the disease. The protective Bordeaux treatment alone at the popcorn stage (March 8) gave only partial control. A second Bordeaux treatment when 50 per cent of the blossoms were open contributed materially towards better control. The question arises, therefore, as to whether or not two protective sprays might be of greater value in preventing blossom infection than one eradicative and one protective spray. The results given here are, of course, insufficient to answer this question.

EFFECT OF ADDED MATERIALS ON THE EFFICACY OF THE ERADICATIVE FUNGICIDE

The importance of increasing the wetting and penetration of the conidial masses by the eradicative preparation was recognized earlier (7). In the present studies sodium lauryl sulfate and emulsifiable petroleum oil (Shell Medium Soluble) were tested for this purpose. Materials such as styrene emulsion (Styralloy), bentonite, and bentonite-sulfur (Kolofog), which might function as adhesives, and ammonium sulfate which is said (2) to activate the herbicidal properties of sodium petachlorophenate, were also included in the trials.

In laboratory tests, detached source twigs bearing sporodochia of *Sclerotinia laxa* were sprayed with the preparations, dried, rewetted with distilled water, placed in a moist chamber, and held at 25° C. Three and 8 days after spraying, 15 sporodochia from each lot of twigs were crushed in separate drops of distilled water to liberate the spores. Sufficient water, usually about 5 ml., was then added to obtain a suspension containing 10 to 25 conidia per low-power microscopic field, and drops of this suspension were placed on the surface of potato-dextrose agar for germination. After 20 hr. at 25° C. the percentage mortality of conidia was determined in the manner outlined by Horsfall (4). Tests made concurrently with these indicated that the sodium pentachlorophenate, which covered the sporodochia and which consequently was brought into the conidial suspension when the sporodochium was crushed, increases mortality of conidia about 4 per cent. Accordingly the germinability data were adjusted to compensate for this effect.

In field trials the different preparations were applied to duplicate plots of trees, and records on sporodochial numbers were taken at the blossoming period.

Table 4 summarizes the results from three laboratory and two field tests. Significant improvement in the eradicative action of sodium pentachlorophenate accompanied the addition of sodium lauryl sulfate and emulsifiable petroleum oil, whereas the other materials apparently varied in this respect. Thus, in laboratory tests, mortality after 3 days was higher among conidia receiving the eradicative preparation plus the wetting agent and bentonite or ammonium sulfate, than among those receiving only the eradicative plus sodium lauryl sulfate. Over a period of 8 days, however, neither bentonite nor ammonium sulfate preparations increased the killing power of the preparation. Styrene emulsion, on the other hand, apparently retarded the action of the preparation for the first 3 days after treatment but did not affect its killing power over the 8-day period. The retarding effect of this material was evident in the preparation containing bentonite but not in those containing ammonium sulfate. Results with bentonite-sulfur, however, were inconsistent; though this material had a tendency to hasten the eradicative action of sodium pentachlorophenate, the effect was variable and consequently not statistically significant.

RELATION OF CERTAIN WEATHER CONDITIONS TO THE ERADICATIVE ACTION OF SODIUM PENTACHLOROPHENATE

A brief summary of the results included in this section has been published (9).

Moisture. In some years, visible discoloration and shriveling of the sporodochia developed soon after the sodium pentachlorophenate preparation was applied to the trees; in other years, not until a week or more later. Eradicative action of the fungicide evidently depended upon the presence

TABLE 4.—*Effect of various supplements on the eradicative action of sodium pentachlorophenate, 5 lb. per 100 gal., against the conidial masses of Sclerotinia laxa on diseased almond twigs*

Supplement, in lb. per 100 gal. or in per cent, added to sodium pentachlorophenate, 5–100 preparation[a]	Mortality of conidia in stated number of days after treatment in laboratory[b]		Reduction[c] of inoculum in field trials	Reduction of blossoming twigs blighted
	3 days	8 days		
	Per cent	*Per cent*	*Per cent*	*Per cent*
No supplement	29	71	65	57
Wetting agent, 0.003 per cent	57	94	83	71
Bentonite, 5–100; wetting agent, 0.003 per cent	81	96	78	83
Bentonite, 5–100; styrene emulsion, 1 per cent; wetting agent, 0.003 per cent	59	82	87	87
Bentonite-sulfur, 5–100; wetting agent, 0.003 per cent	73	97	85	83
Bentonite-sulfur, 5–100; styrene emulsion, 1 per cent; wetting agent, 0.003 per cent	85	87	92	79
Styrene emulsion, 1 per cent; wetting agent, 0.003 per cent	58	97	92	75
Ammonium sulfate, 1.25–100; bentonite, 5–100; styrene emulsion, 1 per cent	89	99	76	74
Ammonium sulfate 1.25–100; wetting agent, 0.003 per cent	79	90	.....	67
Emulsifiable petroleum oil, 0.5 per cent	.....	89	89	77
Difference for significance at 19: 1 odds	21.3	18.4	12.7	13.9

[a] Wetting agent, sodium lauryl sulfate; styrene emulsion (Styralloy); bentonite-sulfur, sulfur fused with bentonite (Kolofog); emulsifiable petroleum oil, a medium oil of 73-sec. viscosity and 92 per cent unsolfonated residue (Shell Medium Soluble).

[b] Detached twigs sprayed, dried, and re-wetted with distilled water were held in a moist atmosphere at laboratory temperatures.

[c] Values reflect both the destruction of sporodochia and repression of their development by the spray.

of moisture in the form of rain or dew, because no visible injury of the conidia occurred in dry weather. At the Locke orchard in 1947, for example (Fig. 1), the inoculum remained unaffected by the fungicide for 5 days after treatment (January 22); but following a rain on January 28, injury ranged from shriveling of a few spores on the ends of the conidial chains to collapse and discoloration of all conidia on the sporodochium. Appar-

ently the fungicide at this time not only destroyed existing conidia, but for a time prevented further development of conidia. It will be noted from figure 1 that heavy rains here preceded new sporodochial development which began in mid-February. Presumably these rains terminated the repressive action of the fungicide.

The ineffectiveness of the 1948 treatments at Arbuckle and Esparto was cited earlier. For about 15 days after these treatments were given (January 14 and 16) the weather was dry; no rain occurred until the Ne Plus Ultra variety was beginning to blossom in early February. Some destruction of inoculum by sodium pentachlorophenate was noticeable after these rains, but apparently not sufficient to effect a material reduction in blossom infection.

To examine further the relation between moisture and toxicity of the sodium pentachlorophenate, detached source twigs bearing numerous sporodochia were sprayed with a 0.5 per cent preparation plus 0.003 per cent sodium lauryl sulfate. Immediately after the spray dried, germinability of the treated conidia was determined and the mortality was 13 per cent. Separate lots of the sprayed twigs were treated as follows: (a) continuously wet, (b) continuously dry, (c) alternately wet and dry at 24-hr. intervals, and (d) sprayed (weathered) for 2 hr. with a fine mist of distilled water, then kept in a moist chamber. All twigs were held at 21° C. for the duration of the experiment. After 5 days, germinability of the conidia was tested with the following results: (a) 89 per cent, (b) 16 per cent, (c) 36 per cent, and (d) 7 per cent mortality, respectively.

The results support the following conclusions drawn from observations in the field: (a) Few conidia are destroyed by the sodium pentachlorophenate within the comparatively short time required for the spray to dry. (b) Dry weather is unfavorable for eradicative action of this material. (c) Under the most favorable moisture conditions, considerable time (probably several days) is required for the fungicide to destroy a high proportion of the inoculum. In the tests, two 24-hr. dry periods alternating with three 24-hr. wet periods reduced the conidium-destroying power of sodium pentachlorophenate materially. (d) Weathering of the spray deposit may render it ineffective before any considerable amount of inoculum is destroyed. In 1947, at the Locke orchard (Fig. 1), the inoculum-repressing effect of the fungicide ceased after heavy rains in early February.

Temperatures. To avoid injury to blossoms and to allow sufficient time for eradicative action, the sodium pentachlorophenate was usually applied a few weeks before the scales of the flower buds separated. Temperatures in mid-January, the time the treatment usually is given, range from near freezing to 12° or 14° C. It seemed desirable, therefore, to determine whether temperature fluctuations within this range influence activity of the fungicide. Accordingly, detached sporodochium-bearing source twigs were sprayed with 0.5 per cent sodium pentachlorophenate plus 0.003 per cent sodium lauryl sulfate, dried, and rewetted with distilled water. Separate

lots of these twigs were placed in moist chambers and distributed among the following temperatures: 5°, 9°, 11°, 16°, 21°, 25°, and 30° C.

To determine the effect of temperature alone on inoculum development, nontreated twigs from which the sporodochia had been removed were placed in moist chambers and held at the above temperatures. In another test, source twigs were removed from orchard trees after being subjected to about 1.5 inches of rain. These also were distributed in moist chambers at the temperatures mentioned above. Judging from the data in figure 3, A, sporodochial development on untreated twigs was highest at 16° C. (60.5° F.). It was not clear, however, whether temperatures above 16° C. were unfavorable to *Sclerotinia laxa* or whether *S. laxa* was adversely affected by other fungi, which were more active at 21° to 30° C. than at 16° C. and below. In any event, the height of inoculum production by *S. laxa* occurs in the spring when the temperature (shaded area in figure 3) does not favor the activity of the other fungi encountered here.

Data on the relation of temperature to destruction of conidia by sodium pentachlorophenate, appearing in figure 3, B, were obtained 5 days after treatment. Mortality of conidia rose as temperature increased from 5° to 30° C. (41° to 86° F.). To determine the effect of high temperatures on subsequent germination of the conidia, nontreated sporodochium-bearing twigs were moistened and placed at 12°, 21°, and 30° C. for 5 days. The percentages of nongerminating conidia were 29, 21, and 26 at the respective temperatures. Apparently, exposure to high temperature for 5 days did not reduce germinability. Immaturity accounted, in part, for the relatively high percentage nongerminability encountered at all temperatures.

The data in figure 3, C, represent the production of conidia on treated and weathered source twigs held at the various temperatures. Judging from the similarity between curves A and C, the sporodochium-repressing effect of sodium pentachlorophenate was largely destroyed by the weathering treatment.

TESTS OF VARIOUS OTHER MATERIALS

In addition to sodium pentachlorophenate, 21 materials were tested in the course of this work. Tests consisted in determining the ability of the preparations to destroy the conidial masses. Different lots of detached source twigs bearing sporodochia of *Sclerotinia laxa* were sprayed with the preparations, dried, rewetted with distilled water, placed in separate moist chambers, and held at 25° C. Three and 8 days after spraying, 15 sporodochia were removed from each lot of twigs. These were crushed in separate 5-ml. lots of distilled water to separate the conidia. The number of conidia in these suspensions commonly ran from 10 to 25 per low-power field of the microscope, but where the conidial numbers were higher than this, distilled water was added. Drops of the suspensions were placed on the surface of potato-dextrose agar in Petri plates and held at 25° C. for 15 to 20 hr. The percentage mortality of conidia was determined in the manner outlined by Horsfall (4).

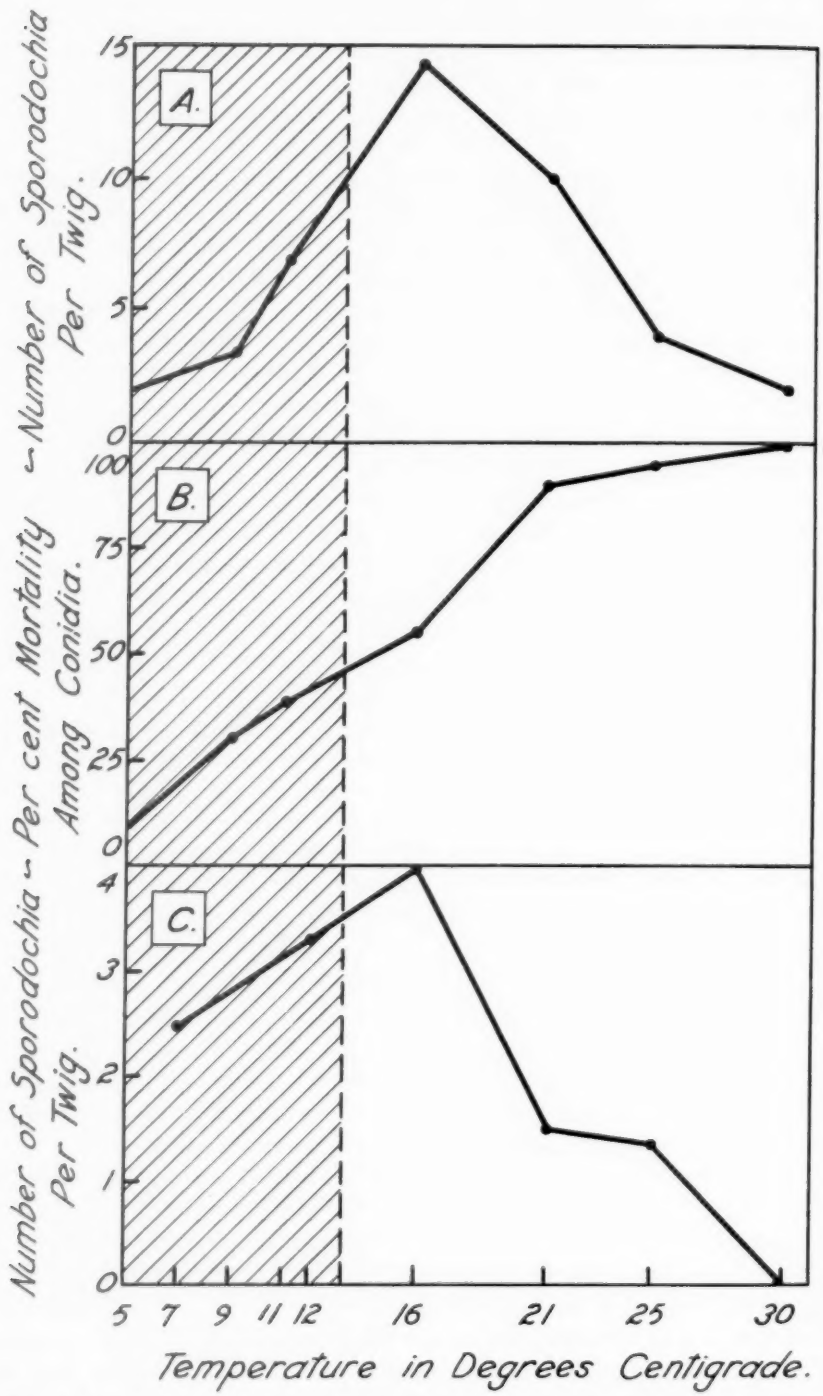

FIG. 3. A. Relation of temperature to production of sporodochia by *Sclerotinia laxa* on almond twigs which received no eradicative spray. B. Relation of temperature to destruction of the conidial inoculum of *S. laxa* by sodium pentachlorophenate. C. Relation of temperature to production of sporodochia on sodium pentachlorophenate-treated almond twigs subjected to 1.5 inches of rain. Shaded area covers the range of temperature which may occur between the time of giving the eradicative treatment and the blossoming period of the host.

In three tests the following materials at the stated concentrations did not produce significant mortality of the conidia: lauryl isoquinolinium bromide, 0.2 per cent; lauryl pyridinium bromide, 0.2 per cent; dimethyl dilauryl ammonium chloride, 0.2 per cent; benzyl lauryl dimethyl ammonium bromide, 0.2 per cent; Para-diisobutyl phenoxy ethyoxyethyl benzyl dimethyl ammonium chloride, 0.25 per cent; disodium ethylene bis dithiocarbamate, 0.75 per cent; zinc dimethyl dithiocarbamate, 0.75 per cent; 2,3-dichloro-1,4-naphthoquinone, 0.5 per cent; trichlorobutane, 2 and 1 per cent; ammonium sulfamate, 2 and 1 per cent; sodium chlorate, 1 per cent; tetramethyl thiuram sulfide, 1 per cent; dichloropropane-dichloropropene, 2 and 1 per cent; phenyl mercuric acetate, 0.1 and 0.05 per cent; dinitro capryl phenyl crotonate in isopropanol, 0.5 and 0.25 per cent; and dinitro capryl phenyl crotonate in oil, 0.5 and 0.25 per cent.

In similar tests the following preparations destroyed from 56 to 75 per cent of the conidia on the sporodochia: phenyl mercuric triethanol lactate, 0.12 per cent; ammonium dinitro-ortho-secondary butyl phenate, 0.24 per cent; and dinitro ortho-secondary-butyl phenol in methanol, 0.12 per cent. These three materials, however, proved relatively ineffective in orchard trials, where repression of inoculum development is an important requisite to eradicative effectiveness.

Neither calcium pentachlorophenate nor zinc pentachlorophenate proved as effective as sodium pentachlorophenate in one orchard test conducted in 1948.

DISCUSSION

On the whole, definite reduction in brown-rot blossom blighting followed spraying of almond trees with sodium pentachlorophenate. Although the degree of control thus obtained compared favorably with that given by a single pre-blossom (popcorn stage) treatment of Bordeaux, one would hardly be justified in relying on the eradicative treatment alone. Bordeaux itself, in a single pre-blossom treatment, was not always satisfactory. Two factors —the large amount of conidia which *Sclerotinia laxa* normally produces in almond trees, and the incompleteness in fungicidal coverage attending the spraying of rapidly unfolding blossoms—probably contribute most towards the uncertainties of control with the standard protective Bordeaux spray. By reducing the amount of conidia in the tree, the eradicative treatment diminished the effect of the first of these two factors.

Whether or not a spray program consisting of one dormant-stage application of sodium pentachlorophenate followed by one pre-blossom Bordeaux treatment is more effective than a program consisting of two pre-blossom Bordeaux treatments, cannot be determined from the results of the single trial reported herein. It is probably not amiss, however, to note that the eradicative treatment required much less exact timing than the protective treatment. This is a distinct advantage in large orchards where several days are required to complete one treatment. In some seasons, such as 1949,

rains interfere with spraying operations to such an extent that two protective treatments cannot be given during the critical period.

SUMMARY AND CONCLUSIONS

A spray preparation consisting of sodium pentachlorophenate (4 lb. per 100 gal.) plus a small amount of wetting agent, applied to dormant almond trees in January, reduced by 37 to 87 per cent the number of conidial tufts (sporodochia) produced by *Sclerotinia laxa* on twigs and adhering blossom parts blighted the previous spring. The reduced amount of blossom infection attributable to the eradicative treatment corresponded roughly to the decrease in inoculum and compared favorably with the reduction in the amount of blossom blighting incident to the application of one protective Bordeaux spray at the popcorn stage of host development. Neither the eradicative treatment nor the protective treatment alone satisfactorily controlled the disease under all conditions. The former reduced the amount of blossoming-twig blight by 31 to 73 per cent in different years, and the latter by 48 to 64 per cent. When trees were given both treatments, however, the disease was reduced 76 to 95 per cent.

In 1949, the eradicative sodium pentachlorophenate treatment followed by a protective treatment of Bordeaux 10–10–100 at the popcorn stage reduced infection 76 per cent; whereas a protective treatment of Bordeaux 10–10–100 at the popcorn stage followed by a Bordeaux treatment when 50 per cent of the blossoms were opened reduced infection 82 per cent.

As in earlier studies, the eradicative action of sodium pentachlorophenate was evident in two ways: (a) by destruction of conidia present when the treatment was given, and (b) by repression of further conidial development. Apparently both modes of action were greatly influenced by moisture. Destruction of conidia was highest when treated twigs were continuously moist, and lowest when twigs were continuously dry. Artificial weathering of treated twigs with a spray of water diminished the sporodochium-repressing effect of the chemical. Similarly, in the field, destruction of conidia was negligible during dry weather, and the repressing effect of the treatment was lost during heavy rains. Apparently, therefore, the year-to-year variability in effectiveness of the sodium pentachlorophenate treatment was caused by rainfall on the spray film. Another factor influencing the eradicative action of sodium pentachlorophenate is temperature. The material was increasingly toxic to masses of conidia as temperature rose from 5° to 30° C.

Significant improvement in the eradicative action of sodium pentachlorophenate accompanied the addition of sodium lauryl sulfate and emulsifiable petroleum oil, whereas no consistent effects were obtained by adding such materials as ammonium sulfate, bentonite, bentonite-sulfur, and styrene emulsion.

Twenty-one other chemical compounds were tested in the course of these studies. Whereas in the laboratory some of these destroyed 50 to 75 per cent of the conidia on source twigs, in the field, where repression of sporo-

dochial development is a requisite to eradicative action, none proved effective.

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THE STIMULATING EFFECT OF DUNG INFUSION ON THE GERMINATION OF SPORES OF USTILAGO STRIIFORMIS[1]

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The difficulty of obtaining satisfactory and consistent germination of spores has been a limiting factor in the study of *Ustilago striiformis* (West.) Niessel. Very low percentages of infection have been obtained when strains of bluegrass were artificially inoculated with spore suspensions. Comparative trials of different methods of inoculation have led to the conclusion that the principal limiting factor in securing high percentages of infection is the poor germination of the spores used. In an effort to overcome this difficulty a study was made of some of the factors influencing spore germination.

Low percentages of germination of spores of *U. striiformis* were reported by Osner (8), Clinton (3), Duggar (5), Davis (4), and others. Davis (4) tested more than 50 different nutrient solutions, plant decoctions, and chemical solutions, but none of them gave any significant evidence of stimulating germination, although subjecting the spores to the fumes of chloroform and then submerging them in citric acid solution shortened the after-ripening period. He concluded that after-ripening for 240–250 days in a moist chamber at room temperature was necessary for germination; however, the percentage of germination was still relatively small.

Kreitlow (6, 7) made extensive germination tests with spores of *Ustilago striiformis* from *Agrostis alba*. He reported a striking increase in germination after incubation of the spores on moist filter paper at 32° to 35° C. for several weeks. In our hands this method has not been satisfactory. Although percentages of germination have sometimes been high, the results have been erratic and inconsistent.

The time required for spores to germinate, as reported by various workers, has varied extensively. Kreitlow did not mention the time required but spoke of incubating spores for 16 to 20 hr. at 25° C. Thirumalachar and Dickson (11) found 5 to 10 days necessary. The present writers also have observed that without special treatment the spores of the smut from bluegrass seldom germinate in less than 5 days.

Smutted leaves were collected from plants growing in the greenhouse, air dried, and stored at room temperature (approximately 24° C.). For germination tests the diseased leaves were cut into small pieces and mixed with distilled water in a Waring Blendor for 1 min. The suspension was passed through a fine screen to remove leaf fragments and centrifuged twice at 1500 r.p.m. for 5 min., the water being decanted each time and

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replaced by more distilled water. The spore mass at the bottom of the centrifuge tube was used for making spore suspensions for germination tests.

Two methods were used throughout these germination experiments. One was the glass-slide smear method of Thirumalachar (10). In this method, clean glass slides smeared with spore suspensions in distilled water or in special solutions were air dried and inverted over the solutions tested in a closed Petri dish. The other method consisted of a simple spore suspension in water or test solutions in Petri dishes. Numerous chemicals and the influence of various environmental factors were tested with these methods, but only negative results were obtained and the data are not given here.

The solution used most extensively and probably most successfully in germination and growth of smut fungi by early investigators was a sterilized aqueous infusion of horse dung. Brefeld (1, 2) used this "Nährlösung" for the culture of those smuts that formed sporidia, and for germination of the spores of many species of smut which germinated poorly in water. Brefeld believed that manure used as fertilizer promotes germination and further development of smut spores in the soil. In view of Brefeld's success with the "Nährlösung," horse-dung infusion was used to treat the chlamydospores of *U. striiformis* in an effort to secure better germination.

Ten gm. of relatively dry horse dung were suspended in 250 cc. of tap

TABLE 1.—*Germination percentages for spores of Ustilago striiformis in various dilutions of horse-dung infusion*

Time (days)	Dilutions from stock solution						
	1 ×	$\frac{1}{2}$ ×	$\frac{1}{4}$ ×	$\frac{1}{8}$ ×	$\frac{1}{16}$ ×	$\frac{1}{32}$ ×	Check
8	0	0	0	17	0	1–2	1
15	0	0	0	40	40	0	0
23	0	0	0	40	45	0	0
26	0	0	60	60	65	0	0

water. Stirring the suspension and filtering it through cheesecloth produced a coffee-brown infusion, which was used as a stock solution. Dilutions were made with water at strengths of 1 : 2, 1 : 4, 1 : 8, 1 : 16, and 1 : 32. One cc. of prepared spore suspension was added to 10 cc. of each of these dilutions and put into Petri dishes for germination. In the control, 10 cc. of distilled water was used instead of horse-dung infusion. Germination tests were made at room temperature (Table 1).

With this evidence of the stimulating effect of dung infusion on spore germination, the experiment was repeated sufficiently to verify the optimum concentration and to determine that the maximum germination was not reached until 15 to 25 days after subjecting the spores to the dung infusion.

Since horse-dung infusion is usually abundantly contaminated with bacteria, this method is impractical for many purposes. The possibility was studied of pretreating the spores with a manure infusion, washing, and

germinating them under more aseptic conditions. Spore masses from the bottom of the centrifuge tube were transferred to filter paper in a Petri dish, and concentrated horse-dung infusion sufficient to wet the paper and spore mass was added. After different periods of time, a small amount of the treated spores was suspended in distilled water and centrifuged once, and the liquid decanted. A dilute spore suspension was then made in distilled water and put into Petri dishes for germination. After periods of 8, 11, and 15 days the germination was 2, 10, and 55 per cent, respectively.

Pretreatment for 15 days in a concentrated dung infusion stimulated germination to a marked degree. Presoaked spores began to germinate within 5 hr. in distilled water, whereas spores not pretreated required 5 to 8 days. In the earlier experiments, when spores were put directly into diluted horse-dung infusion, the time required for germination was 6 to 8 days, some spores germinating after 26 days. Therefore, by this pretreatment with concentrated horse-dung infusion, not only was the percentage of germination increased, but the time required for germination was greatly shortened.

In view of the promising results obtained with horse-dung infusion, further tests were made using infusions of fresh horse dung, dry horse dung, and fresh cow dung. Spores were collected in the same way as in

TABLE 2.—*Germination percentages for spores of Ustilago striiformis after 26 days' pretreatment with various dung infusions*

Infusion	5 hr.	7 hr.	10 hr.	20 hr.
Fresh horse dung	0	tr[a]	25	50
Dry horse dung	tr	25	50	70
Fresh cow dung	tr	45	70	80

[a] tr indicates that spores had just started to germinate.

earlier tests and the same procedure was used for making the spore suspension and germination test, spores being presoaked in the infusion for 26 days (Table 2). All infusions stimulated spore germination, that of cow dung being more effective than that of either fresh or dry horse dung. In all cases, spores that clumped together and floated on the surface of the water germinated better than isolated or submerged spores. Because of the difficulty of counting the clumped or floating spores, however, only those separate and submerged were chosen for counting; hence the percentage of germination was actually higher than the figures indicate.

Since the dung infusions are teeming with microorganisms, it was of interest to know whether the stimulating effect was due to the immediate presence of the microorganisms or to the chemical composition of the infusion. This question was answered by testing the effect of autoclaved dung infusion (Table 3). The stimulating effect was not dependent upon the presence of microorganisms at the time of presoaking, and the stimulating factor was heat-stable.

Smut spores, probably because of the impermeability of their spore walls, are resistant to prolonged soaking in a 1 per cent copper-sulfate solution. Stakman et al. (9) reported the use of 1 per cent copper-sulfate solution for surface-sterilizing chlamydospores of *Ustilago zeae* in obtaining pure cultures. The writers used the same method with success in isolating *U. striiformis* in culture. In order to determine whether the copper-sulfate solution method can still be applied without injury to spores treated with horse-dung infusion, the following experiment was made.

Chlamydospores from diseased bluegrass leaves were collected and treated with concentrated horse-dung infusion for 2 weeks. One lot of treated spores was placed in a 1 per cent copper-sulfate solution for 24 hr. and another lot for 10 hr.; a third lot without further treatment was used as a check. None of the spores treated with copper-sulfate germinated, but germination in the check began within 6 hr. and reached 70 per cent in 34 hr. The greater copper sensitivity of spores presoaked in dung infusion indicates strongly that the effect of presoaking is to increase the permeability of the spore wall.

TABLE 3.—*Germination percentages for spores of Ustilago striiformis in distilled water after soaking 2 weeks in autoclaved and nonautoclaved horse-dung infusion*

Infusion	5 hr.	7 hr.	10 hr.	12 hr.	24 hr.
Autoclaved	0	20	75	80	85
Nonautoclaved	2	55	95	98	98

A final experiment was made to determine how long chlamydospores will remain viable in a horse-dung infusion when stored at room temperature (24° C.) and in a refrigerator (approximately 2° C.). Spore masses were treated continuously with concentrated horse-dung infusion in Petri dishes for the duration of the experiment. Germination tests were made in distilled water at weekly intervals. Spores stored at 24° C. germinated well (50–70 per cent) for 91 days, when the supply of stored spores accidentally were permitted to become very dry. Spores stored at 2° C. continued to germinate as high as 60 per cent after 105 days and as high as 45 per cent after 212 days, when the experiment was discontinued.

The impermeability of the wall of the chlamydospores to water is no doubt the cause of the failure of the spores to germinate well in distilled water without previous treatment. Any factors that alter the permeability of the spore wall so that it will absorb water more readily would probably stimulate the spore to germinate. Many chemical solutions, nutrient solutions, and even natural organic mixtures such as plant tissue juices, soil extract, etc., have been used for this purpose. Some of these substances have been successfully used for breaking the dormancy of other fungus spores; but none of them have been previously found satisfactory for breaking the dormancy of chlamydospores of *U. striiformis*.

In these experiments, when chlamydospores were placed in dilute horse-

dung infusion, the percentage of germination increased greatly as compared with the check. Germination began in the infusion after 5 to 8 days and continued for more than 26 days. On the other hand, spores pretreated with concentrated horse-dung infusion began to send out germ tubes within 5 hr. in distilled water, as compared to 5 to 8 days for spores not presoaked. The horse-dung infusion probably contains some substance that changes the permeability of the cell wall of the chlamydospores and allows the spore to absorb water more rapidly.

The fact that autoclaved horse-dung infusion also stimulated germination indicates that the stimulating substance is heat-stable. The pH of fresh horse-dung infusion is 7.5. It falls to 7.0 after 24 hr., but on the third day after preparation it returns to pH 7.5, and then maintains the same value for an indefinite time. After autoclaving, the pH value falls to 7.0 and remains unchanged. It seems, therefore, that the pH value of the infusion has no relation to its stimulating effect.

Since the chlamydospores germinated much better in distilled water after pretreatment with concentrated horse-dung infusion, it could be concluded that it was not the nutritional substances in the infusion that caused the increase in percentage of germination. This conclusion is supported by the fact that many nutrient solutions on which the spores will grow well have shown no tendency to increase germination.

The most tenable explanation of the effect of horse-dung infusion treatment is that it alters the permeability of the spore cell wall. Soaking with water alone increased the percentage of germination in some cases but not consistently. This would indicate that part of the effect of treating with horse-dung infusion may be due to its soaking effect alone. But this effect is not due merely to contact with water, because chlamydospores dispersed in water for several weeks germinated very poorly. When spores were presoaked in the dung infusion they did not germinate while they were in mass but germinated quickly on dispersal in distilled water. This difference may be due to an accumulation of respiration by-products from the spores themselves and from some other living organisms that may be present.

The stimulating effect of dung infusions on spore germination suggests that horse and cow dung may be an important factor in the heavier infection of *U. striiformis* often found in pastures in comparison with that found in unpastured bluegrass.

Since spores can be collected and presoaked in dung extracts for several months without injury and will germinate promptly on being dispersed in distilled water, it is feasible to prepare in advance, and to keep on hand, a supply of germinable spores.

All the tests reported here were made with spores produced on the host. Although spores produced in culture were stimulated by the treatment, there was much less germination, the results were very erratic, and further work is needed.

SUMMARY

After many chemical solutions and various environmental factors were tested, it was found that infusions of horse or cow dung were very effective in stimulating germination of spores of *Ustilago striiformis* from bluegrass.

Some of the spores suspended in a dilute dung infusion started to germinate after 5 or 6 days, and additional spores continued to germinate for more than 25 days when 60 to 90 per cent had germinated.

Spores presoaked in mass for 15 days or longer in a concentrated dung infusion and then dispersed in distilled water began to germinate within 5 hr. and reached 60 to 90 per cent germination in 20 hr.

Evidence is presented to show that the stimulating factor is heat-stable and that it is not nutritional in its action. It is concluded that the stimulating effect on germination is due to increased permeability of the spore wall.

Spores can be soaked in a concentrated dung infusion at 25° or at 2° C. for at least 5 months without losing viability and will germinate promptly when dispersed in distilled water. This method of providing a convenient supply of germinable spores should remove one of the limiting factors in the study of *U. striiformis* and probably certain other smut fungi whose spores do not germinate readily.

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CHLAMYDOSPORE GERMINATION IN PHYSIOLOGIC RACES OF TILLETIA CARIES AND TILLETIA FOETIDA[1]

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INTRODUCTION

Spore germination in the bunt fungi, *Tilletia caries* (DC.) Tul. and *T. foetida* (Wallr.) Liro, is influenced by many factors, including temperature, moisture, medium, and age of spores. Many of the published results of the investigation of these factors are not in agreement. For example, the cardinal temperatures for germination have been established at several different levels by various investigators (4, 6, 7, 8, 9, 10, 11, 12, 15, 16, 17). Obviously, such discrepancies lead to confusion. This has emphasized the need for further study on spore germination in *T. caries* and *T. foetida*, especially in relation to the phenomenon of physiologic specialization.

Most of the published information on the biology of the wheat-bunt fungi is based on investigations that did not give full consideration to the implications of physiologic specialization. In view of the persistence of the bunt problem, physiologic races have become increasingly important as a primary consideration in its ultimate control. Consequently, a reappraisal of the fundamental behavior of *Tilletia caries* and *T. foetida*, with emphasis on physiologic races of these species, is necessary to a basic understanding of the bunt problem. The results of such a study are presented here.

PERTINENT LITERATURE

According to Woolman and Humphrey (17), Prevost published the first recorded observations on the relation of temperature to spore germination in *Tilletia* in 1807. He found that the spores germinated in water at 17° to 18° C. in 2½ to 3 days, and that the rate of germination decreased with each lowering of the temperature until at 4° to 5° C. germination required 11 to 12 days. Since that time the relationship between temperature and spore germination in the bunt fungi has been confirmed by numerous workers. Most of them have established the optimum at 16° to 18° C. (6, 10, 11, 17); others have placed it at 10° to 15° C. (9, 15), at 18° to 20° C.

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(4, 7, 16), at 15° to 18° C. (8), and above 20° C. (12). Thus, six temperature ranges have been reported as optimum for spore germination in *T. caries* and *T. foetida*. These same workers reported minimum temperatures for spore germination ranging from 5° to 11° C. (4, 6, 10, 11, 15, 16, 17) and maximum temperatures for their germination ranging from 18° to 36° C. (4, 6, 7, 10, 11, 15, 16, 17). Perhaps the lack of agreement can be explained in part by the results reported by Bressman (2) and Hanna (5). The former found that the spores of some races germinated best in water at 6° C., others at 12° C., and still others at 18° C. Similar results were reported by Hanna (5) who found that at a given temperature the spores of some collections germinate more rapidly than those of others.

Several workers have demonstrated that nutriment exerts an influence on spore germination in *Tilletia caries* and *T. foetida*. Stakman (14) found that, with the exception of soil infusion, nutrient media seemed to have a deleterious effect on germination. Rabien (11) reported that dextrose and cane sugar reduced germination, and no germination was obtained in 0.1 per cent peptone solution within 10 days. Kienholz and Heald (7) likewise demonstrated the unsuitability of rich media for spore germination in the bunt fungi. Plain water agar and soil extract agar (2 per cent agar) were the best of six media that they tested for germination. Whether this holds true for all races has not been determined.

Longevity in spores of the bunt fungi is an important factor in bunt control. Woolman and Humphrey (16) observed that the spores of *Tilletia caries* retained their viability for 12 years or longer under laboratory conditions, and Lobik and Dahlstrem (8) found the spores of both species to be viable after 7 years. Fischer (3) germinated spores of *T. caries* from 18-year-old herbarium specimens, and spores of *T. foetida* from 25-year-old specimens. The time required was 12 and 21 days, respectively. In Olgyay's (10) opinion, the viability of the spores of *Tilletia* is not determined by age alone but may depend also on the source and whether stored in powdered form or in bunt balls. Rabien (11) found a progressive decrease in the rate and amount of germination in spores of the bunt fungi following the beginning of the second year after maturity. In contrast, Ajroldi (1) reported that the viability of the chlamydospores of both species reached its maximum the second year and remained high for the first 5 years. None of the work on which these reports are based took into consideration the problem of physiologic races.

MATERIALS AND METHODS

The chlamydospore material used in these studies represented most of the races of *Tilletia caries* (T-races) and *T. foetida* (L-races) described by Rodenhiser and Holton (13). The spore material had been stored dry in the form of smut balls or smut powder at room temperature for varying periods of time.

Unless stated otherwise, the germination tests were made on 4 per cent

water agar. This gave a firm surface which facilitated distributing the spores evenly over the surface of the agar. Chlamydospores, whether taken from smut powder or smut balls, were added to sterile distilled water in sufficient quantities to form a fairly heavy suspension. From this master suspension, spore material was transferred by means of a sterile pipette to the water agar. The spores were easily distributed over the surface of the agar with a sterile loop and in a few minutes the excess water was absorbed or had evaporated. With this method it was possible to distribute spores fairly rapidly on the agar so that their percentage germination could be determined accurately.

In the germination tests the spores were incubated at various temperatures ranging from 0° to 35° C. The writer did not have the control of the temperature equipment and consequently it was necessary to use the most suitable temperatures that were available. The percentage of germination was based upon counts of at least 100 spores except for data presented in table 1, where counts are the average of estimated percentages in three replications. No attempt was made to determine the percentage of germination after 75 per cent of the spores had germinated, because by then the colonies were too closely intermingled.

To test the effect of moisture content of the medium on spore germination, water agar ranging from 1 to 6 per cent agar was used. Difco dehydrated Bacto potato-dextrose agar was used in tests to determine the effect of richness of the medium on spore germination. In addition to being used at full strength,[3] this medium was modified by adding it in powder form to 2 per cent water agar in amounts ranging from 0.5 per cent to 3.0 per cent.

EXPERIMENTAL RESULTS

Temperature

Temperature influences both the rate and the amount of spore germination in *Tilletia caries* and *T. foetida*. Some physiologic races react more quickly to a given temperature than do others (Table 1). All races germinated more rapidly at the high temperature range of 18° to 20° C. than at the low temperature range of 8° to 10° C. The most uniform rate of spore germination for all races was obtained at the intermediate temperature range of 14° to 16° C. Relative rate of germination, however, was not necessarily the same for all races at all temperatures. For example, at the high-temperature range T-10 germinated more rapidly than did T-11, whereas at the low range the reverse was true. These results suggest, therefore, that some races are adaptable to wide ranges in temperature.

On the basis of the data in table 1, it was possible to group the races, according to time required for maximum spore germination, at three temperature ranges. The five groups listed in table 2 are based on rate of ger-

[3] Forty gm. of the dehydrated mixture per 1000 cc. of water. A liter contained: infusion from 50 gm. dried potatoes; Bacto-dextrose, 20 gm.; and Bacto-agar, 15 gm.

TABLE 1.—*Comparative rates of germination of the spores of 25 races of the bunt fungi at three different temperatures*

Days of incubation	Germination[a] at temperatures indicated of race:																								
	T-races																L-races								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	1	2	3	4	5	7	8	9	10
												18–20° C.													
3	T	1	1	0	0	0	0	0	0	0	0	0	0	0	T	T	1	0	1	T	T	0	2	T	T
4	3	4	3	3	4	4	4	4	3	1	T	T	T	T	3	4	4	4	4	4	2	4	4	1	4
5	4		4	4					4	3	2	4	3	4	4						4			4	
6										4	3		4												
7											4														
												14–16° C.													
3	T	T	T	0	1	1	T	1	T	T	T	T	0	0	T	T	2	1	2	0	0	T	2	T	1
4	4	4	2	1	4	4	4	4	3	1	4	3	2	2	4	4	4	4	4	1	3	3	4	3	4
5			4	4					4	4		4	4	4						4	4	4		4	
												8–10° C.													
6	T	T	0	0	T	T	T	1	0	0	0	0	0	0	0	0	T	0	0	0	0	0	0	0	T
7	3	1	T	0	4	4	4	4	0	0	T	0	0	0	0	T	1	T	T	0	1	2	2	T	T
8	4	4	2	T		4			T	T	T	0	T	T	1	3	4	2	4	1	4	4	4	1	4
9			4	3					4	1	3	1	1	2	4	4		4		4				4	
10				4						2	4	4	2	4											
11										4			4												

[a] Estimated germination: T = trace—less than 1 per cent; 1 = 1—25 per cent; 2 = 26—50 per cent; 3 = 51—75 per cent; 4 = above 75 per cent.

mination at the low temperature range. At this temperature the most rapidly germinating races, designated group I, required 4 days less time to reach maximum germination than the slowest germinating races, designated group V. At the high temperature range this time interval was 2 days and at the intermediate range, 1 day. Thus the rate of germination for the races in group I exceeds that of the races in group V at all three temperatures.

The relative rates of germination of the races in groups II, III, and IV were not the same at all temperature ranges (Table 2). For example, the races in group IV required 10 days for maximum germination at the low

TABLE 2.—*Number of days required for 75 per cent of the spores of 25 races of bunt fungi to germinate at three different temperatures*

Group	Race	Days for 75 per cent germination at:		
		8–10° C.	14–16° C.	18–20° C.
I	T-5	7	4	4
	T-6	7	4	4
	T-7	7	4	4
	T-8	7	4	4
II	T-1	8	4	5
	T-2	8	4	4
	L-1	8	4	4
	L-3	8	4	4
	L-5	8	5	5
	L-7	8	5	4
	L-8	8	4	4
	L-10	8	4	4
III	T-3	9	5	5
	T-9	9	5	5
	T-15	9	4	5
	T-16	9	4	4
	L-2	9	4	4
	L-4	9	5	4
	L-9	9	5	5
IV	T-4	10	5	5
	T-11	10	4	7
	T-12	10	5	5
	T-14	10	5	5
V	T-10	11	5	6
	T-13	11	5	6

temperature range. For maximum germination, T-11 of this group required 1 day less at the intermediate range and 2 days more at the high range than did the other races. However, at the intermediate temperature range all of the races reached maximum germination within 1 day of each other. At this range, 14 of the races required 4 days and 11 required 5 days for 75 per cent germination. None of the races germinated in less than 3 days at any temperature range.

The spore material used to obtain the results presented in tables 1 and 2 was not of uniform age. Spores of the various races had been collected

over periods of 1 to 5 years. In order to eliminate age as the factor accounting for differences in the germination of the spores of different races, Idaed wheat seed was inoculated with spores of each race in the spring of 1947 and fresh spores were collected when the wheat matured. The data in table 3 were obtained from germination tests with these fresh spores at four temperatures, 5°, 11°, 13.5°, and 19° C. Since the temperatures used were not the same as in the earlier tests, no direct comparisons can be made. Nevertheless, the general relationships of the various races with reference to the speed of germination are the same as those shown in table 1. Certain races, T-7, L-1, L-3, and L-8, may be classed as "fast germinators" while others, T-10, T-11, T-13, and T-14, may be classed as "slow germinators" at each of the four temperatures. However, some discrepancies in the results of the two tests are notable. For example, the spores of race T-13 which were among the slowest to germinate at all temperature ranges in the first test, were slowest with the exception of T-11 at only the highest temperature (19° C.) in this test. At the other three temperatures in this test, germination was the slowest in the spores of T-14. This suggests, therefore, that the time required for spore germination in T-14 decreases with age.

Nutriment and Moisture in the Medium

As noted earlier, several workers (7, 11, 14) have observed a deleterious effect on spore germination by the presence of nutrients in the medium. A test was conducted, at 16° C., to determine whether races may differ in this respect. Three races of each species, representing both "slow" and "fast" rates of germination, were used in this test. Data in table 4 confirm the results of other investigators, and further establish the fact that nutriment was deleterious to all races used in this study, but more so to some races than to others. In most cases the rate of germination was reduced when only ½ per cent of powdered potato-dextrose agar was added to 2 per cent water agar (Table 4). The effect appeared to be greater on the "slow germinators" than on the "fast germinators." For example, there was less than 1 per cent germination in T-11 after 10 days on standard potato-dextrose agar, whereas maximum germination of L-8 occurred on this medium after 9 days. However, very few promycelia gave rise to primary sporidia. Instead there was bizarre branching and bursting of the promycelia similar to that described by Kienholz and Heald (7).

The amount of agar in the medium also has an effect on the rate of spore germination. As shown in table 4, no significant difference was noted when the medium contained 2.5 per cent or less of agar. Above this concentration the rate of germination decreased up to 6 per cent agar, the highest tried. Of the races tested, the deleterious effect was most pronounced in T-11, a "slow germinator" particularly at 16° C. No effect of agar concentration on germination other than a decrease in the rate was noted.

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TABLE 3.—*Percentage of germination in fresh chlamydospores of 25 races of the bunt fungi at four different temperatures*

Days of incubation	Percentage germination at temperatures indicated of race:																								
	T-races																L-races								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	1	2	3	4	5	7	8	9	10
													5° C.												
7	0	0	0	0	0	0	0	0	0	0	0	T	0	0	T	0	0	0	0	T	T	0	T	0	0
8	0	T	0	0	T	T	39	0	0	0	0	T	T	0	T	T	12	T	2	T	T	0	1	0	T
9	T	9	2	0	3	4	75	2	T	0	2	T	T	0	T	8	46	9	54	8	3	T	20	0	20
10	8	46	40	10	14	34		15	9	T	14	7	6	0	9	29	75	65	75	53	21	17	49	7	59
11	41	69	75	66	56	75		58	60	3	27	30	20	2	38	75		75		75	71	48	75	16	75
12	75	75		75	75			75	75	18	75	75	75	5	75						75	75		75	
13										62				16											
14										75				42											
15														75											
													11° C.												
4	T	T	0	0	0	T	T	0	0	0	T	0	T	0	0	0	T	T	T	T	T	T	T	0	T
5	3	54	13	12	24	10	52	T	T	0	2	1	T	0	T	8	30	T	8	2	T	T	15	0	5
6	56	75	75	75	75	75	75	64	66	5	30	22	25	2	50	68	75	75	75	75	42	26	75	16	75
7	75							75	75	75	75	75	75	16	75	75					75	75		75	
8														40											
9														75											
													13.5° C.												
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	T
4	2	55	20	4	17	6	47	4	T	0	T	3	T	0	T	16	75	2	26	8	5	4	45	0	20
5	75	75	75	75	75	75	75	75	75	8	11	38	12	T	50	75		75	75	75	75	52	75	24	75
6										75	75	75	75	24	75							75		75	
7														75											
													19° C.												
4	75	75	42	75	75	75	75	75	66	5	T	34	T	0	68	75	75	75	75	75	52	26	75	75	75
5			75						75	60	T	75	T	10	75						75	75			
6										75	2		4	75											
7											20		45												
8											56		54												
9											60		75												
10											75														

TABLE 4.—*Effect of moisture content and concentration of nutrients on the percentage of spore germination in six races of the bunt fungi*

Days of incubation	Race, kind of medium, and percentage germination												
	Percentage of agar								2 per cent agar plus indicated percentage potato-dextrose agar				Potato-dextrose agar
	1.0	1.5	2.0	2.5	3.0	4.0	5.0	6.0	0.5	1.0	2.0	3.0	
							T-5						
4	75	75	75	75	46	22	12	18	26	T[a]	T	T	0
5					75	75	75	75	75	75	16	T	T
6											20	T	T
7											20	T	3
9											25	T	20
12												T	20
							T-10						
4	54	24	46	20	21	11	17	T	16	0	0	0	0
5	75	75	75	75	75	75	75	75	75	62	60	T	3
6										75	75	3	15
7												12	54
9												45	54
12												45	54
							T-11						
4	24	12	4	5	T	T	0	0	T	T	T	0	0
5	75	75	75	75	38	2	33	T	T	T	T	T	T
6					75	36	45	T	5	3	T	T	T
7						75	58	25	12	9	T	T	T
9							75	75	75	16	5	T	T
12										16	5	T	T
							L-1						
4	75	75	75	75	75	75	75	75	75	75	56	18	12
5											75	42	16
6												68	20
7												75	26
9													75
							L-8						
4	75	75	75	75	75	75	75	62	75	75	44	19	4
5								75			75	75	36
6													48
7													56
9													75
							L-9						
4	75	75	75	75	75	44	34	25	40	26	10	T	T
5						75	75	75	75	75	75	34	13
6												38	34
7												48	34
9												48	34
12												48	34

[a] T = trace—less than 1 per cent.

Age of Spores

The effect of age of spores on germination is an important consideration, especially when different physiologic races are being tested. To measure this effect, the germination of 11-year-old spores of eight races of

Tilletia caries was compared with that of fresh spores of the same races (Table 5). The tests were made on 4 per cent water agar at two temperatures (8° C. and 20° C.). The old spores had been stored both as smut balls and as powdered spores.

In each of the eight races, the rate of germination of the 11-year-old spores was slower than that of the fresh spores at both temperatures, the effect being more pronounced at the lower temperature (8° C.). Furthermore, the reduced rate of germination was more pronounced in some races than in others, as shown by the contrast between T-2 and T-7. Also, the total germination was reduced in races T-1, T-2, and T-6 at 20° C., and in T-6 at 8° C. While the reduced germination at the higher temperature might have been due to the fact that the agar had dried out considerably, this would not explain the reduced germination in T-6 at the lower temperature. In this case only 40 to 50 per cent of the spores had germinated after 22 days although the agar had not dried out noticeably. From these results it seems evident that a difference exists between races with respect to the effect of age on spore germination.

No consistent difference in the rate or amount of germination was noted between the spores stored as smut balls and those stored as spore powder. However, there were some individual exceptions to this rule. In the case of T-2 at 20° C., both the rate and amount of spore germination were lower in the spores stored as smut balls than in those stored as powder. In contrast, the spores of T-6 stored as smut balls germinated more rapidly and in greater amounts than those stored as powder. This was not true at 8° C. with either race. Similar indications and apparent contradictions are exhibited by other races (Table 5). The irregular nature of these results suggests the need for further study.

Spores of five physiologic races collected at different stages of maturity were tested for germination (Table 6). Collections were made 4 days to 2 weeks apart. When the smut balls were collected at an early stage of maturity, they contained a high percentage of hyaline spores. Apparently these spores were not mature for they failed to germinate, whereas the spores which had attained their normal color germinated readily under the proper conditions. It would appear, therefore, that spores of the bunt fungi become germinable immediately on maturity as indicated by spore color. There was no evidence of need for a resting period before chlamydospores of the races used would germinate. In the collections at earlier stages of maturity, spores of all the T-races germinated better than those of the L-races. Positive conclusions cannot be drawn, however, because of the difficulty of collecting spores of the different races at exactly the same stage of maturity.

DISCUSSION

The lack of agreement regarding many phases of the wheat-bunt problem is understandable when the differences between physiologic races of

TABLE 5.—*Effect of age and the manner of storage on the percentage of spore germination in eight races of Tilletia caries*

Days of incubation	Germination at temperatures indicated of race:																							
	T-1			T-2			T-3			T-4			T-5			T-6			T-7			T-8		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
												20° C.												
4	15	0	0	10	0	0	75	T	T	50	T	5	75	T	0	75	T	10	75	T	T	50	0	0
5	75	20	18	75	5	22		60	60	75	60	40		50	50		5	10		75	75	75	75	50
7		50	50		25	40		75	75		75	75		75	75		50	15						75
9		65	50		30	50											60	15						
												8° C.												
6	T	0	0	5	0	0	T	0	0	T	0	0	T	0	0	0	0	0	T	0	0	0	0	0
8	40	0	0	25	0	0	75	0	0	34	0	0	8	0	0	40	0	0	25	0	0	5	0	0
9	75	0	0	50	0	0		0	0	75	0	0	50	0	0	75	0	0	75	0	0	75	0	0
10		T	T	75	0	T		5	5		2	T	75	T	T		0	0		T	T		T	T
11		5	5		T	10		12	20		10	3		5	5		T	T		27	10		30	25
14		40	40		15	15		75	75		75	50		75	50		10	10		75	75		75	75
22		75	75		75	75						75			75		50	40						

Spores were collected: a—1947; b—1936 (stored as smut balls in vials); c—1936 (stored as spore powder in vials).

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TABLE 6.—*The percentage germination of spores of different physiologic races of Tilletia caries and T. foetida collected at different stages of maturity*

Days of incubation	Race, stages of maturity,[a] temperature, and percentage germination																									
	T-5					T-10					T-11					L-1					L-8				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
											11° C.															
5	T	T	0	0	0	T	0	0	0	0	0	0	T	0	T	T	T	T	3	4	0	5	20	15	14
6	28	37	17	49	42	10	11	T	T	15	5	9	48	34	26	5	13	23	46	75	9	70	75	75	75
7	28	37	57	75	75	30	43	46	62	75	38	52	75	75	75	5	13	23	46		9	70			
8	28	37	57			30	43	75	75		38	52				5	13	23	46		9	70			
											13.5° C.															
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	T	T	T
4	12	3	0	17	2	T	0	0	0	0	T	0	0	0	0	T	6	3	24	20	0	14	32	5	15
5	26	30	75	75	75	6	14	20	10	62	T	T	10	9	25	12	40	48	52	75	6	60	75	75	75
6	26	30				16	50	60	75	75	5	8	66	54	75	12	40	48	52		6	60			
7	26	30				16	50	60			29	37	75	75		12	40	48	52		6	60			

[a] Spores were collected (1947): 1—June 11 (smut balls about pinhead size); 2—June 16; 3—June 20; 4—June 24; 5—July 8 (smut balls almost mature size).

the bunt fungi are considered. Investigators working under identical conditions but with different races may get contradictory results.

The results presented here show clearly that there is a difference between certain of the 25 races in the rate of spore germination. Some races are relatively "fast germinators"; the spores of T-5 germinated faster than those of T-10 or T-13 at all of the temperatures used. In contrast, certain races are "slow germinators," but the majority germinate at a medium rate.

At no one temperature did the spores of all races germinate at the same time. The nearest approach to this was at a temperature range of 14° to 16° C. At this range only 24 hr. elapsed from the time 75 per cent of the spores of the fastest germinating race had germinated until an equal percentage of the spores of all the other races had germinated. At temperatures above and below this range the differences in the times required for germination of the various races became progressively greater. Possibly some races have an optimum temperature for spore germination above 14° to 16° C. and others below this temperature. However, this temperature range is close to the optimum for all races. Hence, at this range the rate of spore germination in any race is decreased but little because of temperature.

The exact optimum temperature for spore germination in the various physiologic races was not determined in this study. However, the results obtained indicate clearly that no one temperature is optimum for all the races. For example, at 19° C., 3 to 4 days longer were required for maximum germination of races T-11 and T-13 than for T-14. The reverse of this was true at temperatures of 5°, 11°, and 13.5° C. At 5° C., 3 days longer were required for T-14 than for T-11 and T-13. Possibly the optimum temperature for spore germination in races T-11 and T-13 is 12° to 14° C. and that of T-1 about 18° C. This would suggest a range of 5° to 6° C. in the optimum temperatures for spore germination of the 25 races of the common bunt fungi.

Nutrition level is an important factor in the germination of *Tilletia caries* and *T. foetida* spores. The most rapid germination occurred on a medium containing 2.5 to 3 per cent agar. The rate of germination decreases at the high agar content possibly because less water is immediately available to the spores. In these investigations, 4 per cent water agar was used in most instances for the spore germination tests. There was no more difference in the relationships of the various races at this agar content than at any of the others tried. In general, a nutrient-containing medium should not be used. There was, however, some difference in the capacity of races to germinate on such media.

Physiologic races of the bunt fungi differ in many respects and, since no two races behave exactly the same under all conditions, it manifestly would be difficult for one worker to duplicate the results of another. Such things as the race, the temperature, the age of spores, and the medium used

must be taken into consideration. Age up to 5 years apparently had little effect on the amount or rate of germination of spores in the races tested, but there was a pronounced retardation of germination in spores 11 years old, being more pronounced in some races than in others. Obviously, from these studies, it is unwise to generalize broadly on the basis of results obtained with one race, or even with several races under limited conditions of experimentation. In other words, the phenomenon of physiologic specialization in *Tilletia caries* and *T. foetida* is a matter for major consideration in dealing with any wheat-bunt problem.

SUMMARY

Physiologic races of the wheat-bunt fungi differ widely in their temperature requirements for spore germination. The optimum temperature for certain races differed by as much as 5° C. Some races are "fast germinators" and others "slow germinators" at all temperatures used in this study. No one temperature is optimum for all races and the rate of spore germination for all races is not the same at any one temperature.

On a nutrient-free medium containing over 3 per cent agar, the rate of spore germination was decreased. A small amount of nutrient in the medium also decreased the rate and amount of germination. The degree to which these effects were expressed differed among the races tested.

The rate and amount of germination in 11-year-old spores was markedly less than that for fresh chlamydospores, especially in some races. There was no difference in fresh spores and in those 4 to 5 years old. Immature smut balls having many hyaline spores gave a low percentage of spore germination, this being more pronounced in some races than in others.

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A GENETIC STUDY OF HALO BLIGHT REACTION IN PHASEOLUS VULGARIS[1]

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Resistance to halo blight caused by *Pseudomonas medicaginis* var. *phaseolicola* (Burkh.) Stapp and Kotte in beans (*Phaseolus vulgaris* L.) has been reported previously from this laboratory by Jensen and Goss (3). While inheritance studies have been made on the bean plant, they have failed to include halo blight reaction. It would be an advantage for the plant breeder to possess information concerning the mode of inheritance of factors governing the reaction to this disease. The number of genes involved and the linkage relations of such genes with other genes have a direct bearing on the breeding method to be followed.

The genetic work was begun in 1946 using two resistant parents and two susceptible parents for this study. Much of the data is dependent upon the F_2 generation since good infection in the field using F_3 progeny was difficult to obtain because of unfavorable weather conditions.

A total of 1,667 F_2 plants have been classified for halo blight reaction. These plants have also been classified as to pod type, growth habit, and blossom color to determine possible linkage relation with disease reaction. Only one cross in the F_1, totaling 87 plants, has been studied as regards resistance to halo blight.

EXPERIMENTAL METHODS

The parents selected for this investigation were the resistant Mexican Red and Arikara Yellow varieties and the susceptible Asgrow Stringless and U. S. No. 5 Refugee varieties. Arikara Yellow, an Indian variety, and Mexican Red are early runner varieties with white blossoms, stringy pods, and indeterminate habit of growth. The Asgrow Stringless variety has a determinate growth habit with stringless pods and vinaceous-lilac blossoms. The U. S. No. 5 Refugee parent possesses stringless pods, pink flowers, and has a semideterminate growth habit.

Methods of growing the bacterial organism in culture and of applying the inoculum were developed prior to 1946 in connection with testing varieties for their reaction to the blight (2). Twenty-four-hour cultures were used for inoculation and were grown on media consisting of potatoes, 300 gm.; peptone, 5 gm.; sodium phosphate, 2 gm.; sodium chloride, 2 gm.; sodium citrate, 1 gm.; dextrose, 6 gm.; agar, 17 gm.; and asparagine, 1 gm. in 1 liter of water. They were grown in test tubes or in Duraglass bottles. The concentration of the inoculum was roughly standardized by using a

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bacterial suspension that was slightly turbid. This concentration was obtained by using the growth on a test tube slant in 2 liters of water.

This paper deals with experiments involving the use of halo blight culture listed as Plant Pathology Accession No. 2076. This culture was reisolated every season prior to routine inoculations. Later work will entail use of other available isolates.

The method of applying the bacterial suspension was similar in principle in the field and greenhouse experiments. A Hudson electric sprayer with a fine nozzle was used in the greenhouse; the inoculated plants were placed under a muslin tent and the humidity kept high by means of a humidifier. In the field a 4-gal. Hudson sprayer was employed, the inoculum being applied at 4 p.m. Field inoculations were in all cases made on plants grown in furrow irrigation at the Scotts Bluff Substation, Mitchell, Nebr. The most favorable age of plant for inoculation had been determined previously in varietal tests (2). The most opportune time for infection was during the unfolding of the third trifoliate. Because of variation of segregating populations, the plants were inoculated on three successive days.

The reaction of the parental varieties was known from previous tests; also methods of handling large numbers of bean plants in the greenhouse were to some extent standardized before the genetic study began (3). In all larger samples representatives of each parent were inoculated at the same time as their hybrid populations. The plants were grown individually in 5-in. clay pots in composted soil. All seed planted in the greenhouse was scarified before planting to insure a uniformly high percentage of germination. The F_1 plants were grown under wire cloth in the field to protect them from hail, and with individual care many of these produced large amounts of F_2 seed. A large number of inoculated F_2 plants in the field and greenhouse produced F_3 seed.

The system of classification as to infection type consisted of two classes described as follows: R = resistant—small, necrotic, haloless lesions on the leaves, occasionally small, chlorotic areas surrounding the necrotic spots. S = susceptible—large lesions with halos, occasionally a mixture of halo and haloless lesions on the same leaf.

Figure 1 includes examples of the reaction types. Plants grown in the greenhouse were classified on the 15th day after inoculation, but often were observed over a longer period. In testing for Mendelian segregation, the plants with small, chlorotic areas surrounding the lesions were included in the resistant class and the plants with a mixture of halo and haloless spots were considered susceptible. Goodness of fit of the observed to the theoretical ratios was tested statistically by means of the chi-square (χ^2) test.

Linkage relations of disease reaction and other characters received attention. Data were obtained for inheritance of pod type, growth habit, and blossom color. Blossom color is a valuable criterion in judging the presence, or absence, of contamination in crosses. The other characters are important from a horticultural standpoint.

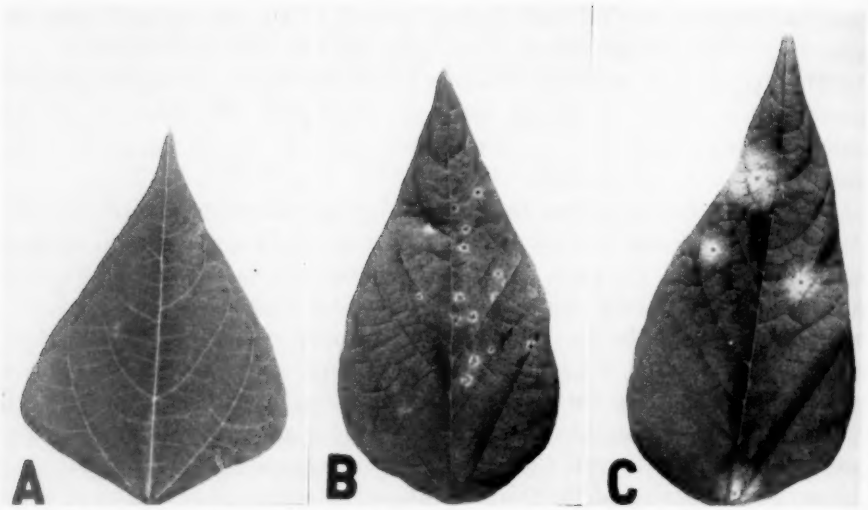

FIG. 1. Infection types produced by halo blight culture No. 2076 on beans. A. Haloless lesions. B. Lesions with restricted halos. C. Lesions with large halos.

RESULTS

Inheritance of Resistance

Mexican Red × U. S. No. 5 Refugee. In the greenhouse tests in 1946–1947 the 104 F_2 plants from a cross of Mexican Red × U. S. No. 5 Refugee were inoculated with halo blight. This cross segregated in the F_2 generation in the ratio of 19 resistant to 85 susceptible. The reciprocal cross segregated in the F_2 generation in the ratio of 25 resistant to 109 susceptible. These results from the two crosses indicated a single major gene governing halo blight reaction with susceptibility dominant ($\chi^2 = 2.51$ and 2.88) (Table 1). The F_3 families from these F_2 plants were tested in the

TABLE 1.—*Inheritance of resistance to halo blight in F_2 generations of the crosses resistant × susceptible or the reciprocal*

Cross	Location of test	Observed segregation	χ^2 for ratio indicated[a]
Mexican Red × U. S. No. 5 Refugee	Greenhouse	85 S : 19 R	2.51 (3 : 1)
U. S. No. 5 Refugee × Mexican Red	Greenhouse	109 S : 25 R	2.88 (3 : 1)
Mexican Red × U. S. No. 5 Refugee	Field	34 S : 16 R	1.31 (3 : 1)
U. S. No. 5 Refugee × Mexican Red	Field	38 S : 14 R	0.10 (3 : 1)
Combined		266 S : 74 R	1.90 (3 : 1)
Arikara Yellow × U. S. No. 5 Refugee	Greenhouse	57 S : 41 R	14.82 (3 : 1)
U. S. No. 5 Refugee × Arikara Yellow	Greenhouse	70 S : 32 R	2.21 (3 : 1)
Arikara Yellow × U. S. No. 5 Refugee	Field	101 S : 22 R	3.32 (3 : 1)
U. S. No. 5 Refugee × Arikara Yellow	Field	108 S : 29 R	1.07 (3 : 1)
Combined		336 S : 124 R	0.94 (3 : 1)
Mexican Red × Asgrow Stringless	Greenhouse	258 S × 171 R	2.64 (9 : 7)
Asgrow Stringless × Mexican Red	Greenhouse	167 S × 161 R	3.80 (9 : 7)

[a] Level of significance at 5 per cent point is 3.841.

field in 1947 but environmental conditions decimated the stand which resulted in an insufficient number for adequate data.

The F_2 progeny from the Mexican Red × U. S. No. 5 Refugee cross and reciprocal were again tested the following year in the field and found to segregate in ratio substantiating the first test (χ^2 for 3 : 1 = 1.31 and 0.1). This indicated that one major factor governed the reaction to halo blight. The combined total chi-square value for the two tests was 1.90 (Table 1).

Arikara Yellow × U. S. No. 5 Refugee. The susceptible parent, U. S. No. 5 Refugee, was crossed with a resistant parent, Arikara Yellow. The F_2 progeny from this cross and its reciprocal were subjected to artificial inoculation with halo blight. In 1946–1947 the F_2 plants segregated into the ratio of 70 susceptible to 32 resistant plants (χ^2 for 3 : 1 ratio = 2.21). The ratio for the F_2 generation of the reciprocal cross was 57 susceptible to 41 resistant, which would indicate a good fit for a 9 : 7 ratio ($\chi^2 = 0.15$). A distorted ratio of the reciprocal cross in the 1946–1947 test may have been caused by poor infection.

In the 1948 field tests F_2 progeny of the same cross, Arikara Yellow, U. S. No. 5 Refugee, and the reciprocal, segregated in a ratio of 101 susceptible to 22 resistant and 108 susceptible to 29 resistant, respectively. These data (Table 1) indicate a good fit for a 3 to 1 ratio. The combined total for the greenhouse and field tests indicates a single factor difference (χ^2 for 3 : 1 = 0.94).

Mexican Red × Asgrow Stringless. The reaction of the F_2 generation of the Mexican Red × Asgrow Stringless cross and reciprocal to halo blight indicated resistance dominant over susceptibility although a few plants showed a few halos present. Of 97 F_1 plants tested in the greenhouse, two showed chlorotic areas on their leaves comparable to those produced on the susceptible Asgrow Stringless. The remainder showed a high degree of resistance. The results from inoculation of F_1 plants did not conform to those obtained with the F_2 generation. The F_1 plants were inoculated during flowering time to prevent killing by the disease. The F_2 generation was inoculated during the unfolding of the third trifoliate. Similar reaction on F_1 plants to halo blight was obtained by Jensen and Livingston, unpublished data, indicating that age of plant affects resistance.

The F_2 generation of the Mexican Red × Asgrow Stringless cross was tested in the greenhouse the following year, 1947–1948. These segregated in the ratio of 171 resistant to 258 susceptible. The F_2 progeny of the reciprocal cross appeared to segregate similarly with 161 plants resistant to 167 susceptible, again indicating a good fit for a 9 : 7 ratio (Table 1).

Linkage

Association or independent inheritance of various plant characters and halo blight was studied. Characters considered were stringless versus stringy pods, determinate versus indeterminate growth habit, and flower color.

Flower color and resistance. Flower color was studied because it aids in determining the presence or absence of selfs and would be a very usable character in case linkage was found. Analysis of the F_2 data of the Mexican Red × U. S. No. 5 Refugee cross and its reciprocal revealed that a single gene pair governed flower color. The chi-square values of 0.25 and 1.03 for the greenhouse and field tests, respectively, indicate a good fit for a 3:1 ratio for flower color, pink being dominant over white. These results conform with those of Johannsen (5) who found that one factor governed flower color. Shaw (8) and Shaw and Norton (9) reported that blossom color was due to a single gene.

Upon inspection the data from the greenhouse and field test indicated a 9:3:3:1 F_2 ratio involving flower color and halo blight reaction. Chi-square values of 0.66 and 0.773 for independence showed that flower color and disease reaction were independently inherited.

Flower color of another cross involving Arikara Yellow and U. S. No. 5 Refugee indicated a single factor difference, the F_2 progeny segregating 186 pink and 55 white flowers. The chi-square value was 0.61 which is indicative of a good fit for a 3:1 ratio.

Data from the Arikara Yellow × U. S. No. 5 Refugee cross yielded a 9:3:3:1 F_2 ratio for the two characters, flower color and halo blight reaction, each being governed by a single gene. The characters were apparently not linked.

Pod type and resistance. The relationship between the stringy pod character and halo blight reaction was studied in the inheritance tests. Two complementary genes were shown to govern the stringy pod character in the cross Mexican Red (stringy) with U. S. No. 5 Refugee (stringless). The chi-square values of 1.35 and 0.09 for the cross and its reciprocal, respectively, indicate a good fit for a 9:7 ratio with the stringless type dominant. In the Arikara Yellow × U. S. No. 5 Refugee cross and reciprocal, a single factor difference was found for pod type. Prakken (7) found 15 stringless to 1 stringy in the F_2 generation. Currence (1) observed two distinct types of stringiness of pods, one due to two dominant complementary genes, the other to an incompletely dominant factor for stringlessness with an inhibiting gene. Joosten, according to Wade (10), differentiated 10 classes of stringiness. These divergent results need further investigation for adequate interpretation.

Independent inheritance between stringiness of pods and disease reaction was investigated; results are in table 2.

The chi-square values of 4.671 and 0.994 for the Mexican Red × U. S. No. 5 Refugee and Arikara Yellow × U. S. No. 5 Refugee crosses and their reciprocals indicated that the factors for resistance to halo blight and pod type were not associated in inheritance.

Growth habit and resistance. Inheritance of determinate and indeterminate habits of growth was also investigated in conjunction with disease readings. The F_1 generation from Mexican Red (indeterminate) × Asgrow

TABLE 2.—*Chi-square test for independence and goodness of fit of halo blight reaction and the stringy pod character of the F_2 generation of the crosses Mexican Red × U. S. No. 5 Refugee and Arikara Yellow × U. S. No. 5 Refugee*

Cross	Phenotype[a]				χ^2	
	DS	dS	Ds	ds	Independence[b]	Ratio indicated[c]
Mexican Red × U. S. No. 5 Refugee	42	24	6	10	3.624	4.671 (27:21:9:7)
Arikara Yellow × U. S. No. 5 Refugee	134	52	38	10	0.994	3.793 (9:3:3:1)

[a] D and d refer to stringless and stringy pod, respectively; S and s to susceptible and resistant types.
[b] Level of significance at 5 per cent point with 1 degree of freedom equals 3.841.
[c] Level of significance at 5 per cent point with 3 degrees of freedom equals 7.815.

Stringless (determinate) indicated complete dominance of the indeterminate growth habit. This was clearly substantiated by a definite segregation in the F_2 into indeterminate and determinate habit with a ratio approaching 3:1. The chi-square values for the Mexican Red × Asgrow Stringless and reciprocal cross were 1.36 and 2.54 respectively, indicating a good fit for single factor difference. Norton (6) obtained similar results with a single factor difference for growth habit, indeterminate being dominant over determinate.

The possible association of the disease reaction with growth habit was studied in the cross Asgrow Stringless × Mexican Red. Independent assortment of the two factors would produce segregation in the ratio: 27 susceptible, indeterminate; 21 resistant, indeterminate; 9 susceptible, determinate; and 7 resistant, determinate. The F_2 progeny segregated in the ratios of 124, 108, 42, 52 for the Asgrow Stringless × Mexican Red cross and 173, 122, 78, 40 for the reciprocal cross. The analysis of these data is shown in table 3. The chi-square for Asgrow Stringless × Mexican Red was 9.27 and for the reciprocal 8.81. Combined, the chi-square was 5.37, indi-

TABLE 3.—*Chi-square test for independence and goodness of fit of halo blight reaction and growth habit of the F_2 generation of the cross Mexican Red × Asgrow Stringless or the reciprocal*

Cross	Phenotype[a]				χ^2	
	SP	sP	Sp	sp	Independence[b]	For 27:21:9:7 ratio[c]
Mexican Red × Asgrow Stringless	173	122	78	40	1.966	8.796
Asgrow Stringless × Mexican Red	124	108	42	52	2.057	9.253
Combined	297	230	120	92	0.004	5.367

[a] S and s refer to susceptible and resistant types, respectively, P and p to indeterminate and determinate plants.
[b] Level of significance at 5 per cent point with 1 degree of freedom equals 3.841.
[c] Level of significance at 5 per cent point with 3 degrees of freedom equals 7.815.

cating independent assortment. No linkage was shown when the cross and its reciprocal were considered as separate units or combined as indicated by chi-square for independence (table 3).

DISCUSSION

This paper is a progress report on the mode of inheritance of resistance in beans to the halo blight organism and the association of resistance with other desirable plant characters.

It is impossible to draw conclusions that would include all bean varieties. In the first instance, only four out of more than 200 bean varieties were tested, although the selected parents represented typical reactions of the susceptible and resistant classes. Secondly, only one culture or strain of the causal agent of halo blight was employed in this investigation. It was previously found (4) that there are several strains of this bacterium and that they differ in pathogenicity. Although a culture representing a high degree of pathogenicity was used, this does not necessarily mean that other cultures would act in a similar manner. This may be a partial explanation of the variation in the symptom expression of the hybrid populations.

Complex as the disease resistance may be, a factorial explanation is attempted. An analysis was made to determine the major genes involved. The two parents selected as typical for the susceptible class apparently do not possess identical gene pairs governing reaction to halo blight. In one instance U.S. No. 5 Refugee showed a single factor difference upon crossing with the resistant parents, Mexican Red and Arikara Yellow, whereas Asgrow Stringless differed by two factors from the two resistant parents. These results present additional information regarding the danger in making premature conclusions in respect to disease reaction for a single crop following the use of a small number of varieties within a species.

Although only four varieties were tested, an attempt is made to arrive at some interpretation of the genetic complexity of halo blight reaction. The genotypes of the susceptible parent, U.S. No. 5 Refugee, and the resistant parents, Arikara Yellow and Mexican Red, were interpreted as possessing homozygous dominant genes SS for susceptibility and homozygous recessive genes ss for resistance, respectively.

The genetic interpretation using another susceptible variety, Asgrow Stringless, indicated a more complex picture. Segregation of the F_2 progeny of a cross between Asgrow Stringless and Mexican Red suggested a bifactorial inheritance of resistance. Chi-square values proved a good fit for a 9 to 7 ratio of the second filial generation of this cross and its reciprocal. The simplest explanation would be that the susceptible parent possessed two dominant factor pairs, $S_1S_1S_2S_2$ for susceptibility, contrasted with an $s_1s_1s_2s_2$ genotype for Mexican Red. At least two dominant factors must be present for susceptibility to express itself, and for resistance three recessive genes must be resident in the chromosome.

To explain the results from the other cross in the light of this genotypic

deduction would require evidence of the fact that Mexican Red and U.S. No. 5 Refugee both carry a pair of homozygous resistant factors in addition to the recessive and dominant genes located in the respective varieties. A cross of the two susceptible varieties would afford a more accurate explanation of the genetic makeup of the two varieties. Similarly, a cross between the two resistant varieties, Mexican Red and Arikara Yellow, and each resistant with the other susceptible should yield useful information and clarify the genetic composition of the two resistant varieties.

Linkage relations of disease reaction and other plant characters were included in the tests. Flower color was found independently inherited from halo blight reaction in crosses between Arikara Yellow × U.S. No. 5 Refugee and between Mexican Red × U.S. No. 5 Refugee. A single gene governed the flower color in these two crosses.

In studying the relationship of the stringy pod character and halo blight reaction, it was found that these characters segregated independently in crosses of Mexican Red × U.S. No. 5 Refugee and Arikara Yellow × U.S. No. 5 Refugee and their reciprocals. Two complementary genes were found to govern the stringy pod character in the former cross. In the Arikara Yellow × U.S. No. 5 Refugee cross a single factor difference was indicated. Possible linkage of the determinate or indeterminate habits of growth and disease reaction was also considered. The first and second filial generations of the Mexican Red × Asgrow Stringless cross indicated that indeterminate is dominate and that there is no linkage between the two factors. Growth habit was determined by a single gene.

With data obtained under the conditions of the experiment it appears to be possible to develop a resistant plant with desirable pod type and growth habit without much difficulty. Studies will be necessary to determine relationships of other characters as well. In conjunction with these inheritance studies, selections are being made in an effort to produce resistant commercial varieties.

SUMMARY

The mode of inheritance of halo blight of beans (*Phaseolus vulgaris* L.) caused by *Pseudomonas medicaginis* var. *phaseolicola* (Burkh.) Stapp and Kotte and its association with plant characters was studied. One or two factors governed the disease reaction, depending on the susceptible and resistant parents employed, susceptibility being dominant.

The resistant varieties Mexican Red and Arikara Yellow when crossed with U.S. No. 5 Refugee indicated a monofactorial type of inheritance. However, the F_2 progeny from the cross between Mexican Red and the susceptible variety Asgrow Stringless indicated a bifactorial mode of inheritance. The F_1 plants from the Mexican Red × Asgrow Stringless appeared resistant, probably due to age of host tissue.

Halo blight reaction was not associated in inheritance with flower color or the stringy pod character. The determinate or indeterminate habits of

growth were not linked with disease reaction and individuals possessing resistance and desirable growth habit might be obtained without difficulty.

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